UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF PREVENTION, PESTICIDES AND TOXIC SUBSTANCES

July 12, 2001

MEMORANDUM

SUBJECT: Implementation of the Determinations of a Common Mechanism of Toxicity for N-Methyl Carbamate Pesticides and for Certain Chloroacetanilide Pesticides

FROM: Marcia E. Mulkey, Director /s/
Office of Pesticide Programs (7501C)

TO: Lois Rossi, Director
Special Review and Reregistration Division (7508C)

Jim Jones, Director
Registration Division (7505C)

Jay Ellenberger, Acting Director
Field and External Affairs Division (7506C)

On July 10, 2001, I signed two memoranda, each setting out OPP's determination that each group of pesticides – the n-methyl carbamate pesticides and certain of the chloroacetanilide pesticides – shares a "common mechanism of toxicity." OPP will place the memoranda announcing the common mechanism determinations, along with the attachments to the memoranda, in a public docket and will post the memoranda on OPP's website. In addition, OPP will notify its stakeholders of these determinations using the Pesticide Program Update messaging system and will announce the availability of these documents to the media. Further, OPP will invite the public to submit comments on these determinations, as well as any relevant new data or analyses, over the next 60 days. Finally, as OPP moves ahead, including when we conduct assessments of the potential cumulative risk from exposure to these two groups of pesticides, we expect OPP to consider fully all comments and information submitted by the public.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

July 10, 2001

MEMORANDUM

SUBJECT: A Common Mechanism of Toxicity Determination for Chloroacetanilide Pesticides

FROM: Marcia E. Mulkey, Director /s/
Office of Pesticide Programs (7501C)

TO: Lois Rossi, Director
Special Review and Reregistration Division (7508C)

Jim Jones, Director
Registration Division (7505C)

This memorandum summarizes the position of the Office of Pesticide Programs (OPP) with respect to the existence of a common mechanism of toxicity for pesticides belonging to the chemical group identified as chloroacetanilides and, therefore, provides direction to the Special Review and Reregistration Division (SRRD) as it proceeds in conducting the reassessment of the tolerances for these pesticides and to the Registration Division (RD) as it considers any tolerance actions involving these pesticides. The memo describes the information the scientists considered, the process (including external peer review) followed in developing a position, and the conclusions regarding this scientific issue.

Background

The Food Quality Protection Act (FQPA) amended the laws under which EPA evaluates the safety of pesticide residues in food. Among other types of information EPA is to weigh when making safety decisions, the new amendments direct EPA to consider "available information concerning the cumulative effects of such residues and other substances that have a common mechanism of toxicity." Sec. 408(b)(2)(D)(v) of the Federal Food, Drug, and Cosmetic Act. FQPA also directs EPA to apply the new safety standard to tolerances established prior to the passage of FQPA. Further, in carrying out the tolerance reassessment provisions of FQPA, EPA "shall give priority to review of the tolerances or exemptions that appear to pose the greatest risk to public health." Sec. 408(q)(2).

<u>Review of the Chloroacetanilide Pesticides</u>

The chloroacetanilide pesticides represent a class of food use pesticides that have been given high priority by OPP for the reassessment of tolerances in accordance with the mandates of FQPA. The group of chloroacetanilide pesticides covered by this review consists of **Acetochlor**, **Alachlor**, **Butachlor**, **Metolachlor** and **Propachlor**. Various members of this group of chloroacetanilide pesticides have been shown to result in several different types of tumor responses in laboratory animals (e.g., nasal, thyroid, liver, and stomach tumors). Therefore, as part of the reassessment, OPP scientists considered several different potential common mechanism of toxicity groupings for these chemicals.

In reviewing this issue, OPP scientists were guided by several relevant Agency science policies, including *Guidance for Identifying Pesticide Chemicals and Other Substances that Have a Common Mechanism of Toxicity*. [Attachment A]. Additionally, on March 19, 1997, the Agency presented to the FIFRA Scientific Advisory Panel (SAP) a draft case study illustrating the application of the Common Mechanism Guidance to the grouping of chloroacetanilide pesticides based on a common mechanism of toxicity. In its recommendations, the SAP noted that the case study was ". . . excellent, well-presented, and very appropriate." [Attachment C] The SAP agreed with the Agency's conclusion that there is sufficient evidence to support the grouping of certain chloroacetanilides that cause nasal turbinate tumors by a common mechanism of toxicity. Even though the case study illustrated that certain chloroacetanilides had the capacity to cause the development of thyroid follicular cell tumors, the SAP recommended that this endpoint should not be used to support a finding that the chemicals shared a common mechanism of toxicity because the thyroid effects were seen at a maximum tolerated dose (i.e., a treatment dose that alters the animal's longevity or well being because of excessive toxicity).

Following the completion of the SAP's 1997 peer review of OPP's draft document on the grouping of chloracetanilides based on a common mechanism of toxicity, and conclusions therein, OPP conducted a "Peer Review Self-Assessment and Verification Survey." [Attachment D] In the survey, OPP agreed with the conclusions of the SAP.

Upon consideration of the SAP comments, OPP's own reviews and the data underlying these reviews, as well as additional information received by the Agency from registrants or presented in the open literature since the 1997 draft document, OPP has revised its science document discussing the potential grouping of chloroacetanilide pesticides, or a subgroup of them, based on a common mechanism of toxicity. [Attachment B] OPP has determined that the information received after 1997 reinforces its earlier conclusions. Thus, in the revised document entitled *The Grouping of a Series of Chloroacetanilide Pesticides Based on a Common Mechanism of Toxicity*, OPP has concluded that only some of the pesticides that comprise the class of chloroacetanilides should be designated as a "Common Mechanism Group" based on the development of nasal turbinate tumors by metabolism to a highly tissue reactive moiety, i.e., quinoneimine. [Attachment B]. Thus, only Acetochlor, Alachlor, and Butachlor should be grouped based on a common mechanism of toxicity for nasal turbinate tumors. Although Metolachlor does distribute to the nasal turbinates, and might produce a quinoneimine, it is not apparent from currently available data that it shares the same target site in the nasal tissue as Acetochlor, Alachlor and Butachlor. Although Propachlor does produce a precursor of a quinoneimine, the available data do not support its tumorigenicity to the nasal turbinates.

Acetochlor, Metolachlor, and Propachlor produce liver neoplasia in rodents. Additionally, Butachlor and Alachlor, and possibly Acetochlor (at high doses), induce stomach tumors. At this time, however, there is inadequate data for grouping chloroacetanilides based on a common mechanism of action for either the induction of stomach or liver tumors. [For a more detailed discussion, see Attachment B.] Although the available evidence supports potentially grouping Acetochlor, Alachlor, and Butachlor by a common mechanism of toxicity based on the induction of thyroid follicular cell tumors, as stated above, this endpoint is not suitable for risk assessment because the thyroid effects were noted at excessive toxic doses. [For a more detailed discussion, see Attachment B.]

Conclusions

In conclusion, it is OPP's position, at this stage in the tolerance reassessment process, that only some chloroacetanilides, namely **Acetochlor, Alachlor, and Butachlor should be considered as a Common Mechanism Group** due to their ability to cause nasal turbinate tumors. For purposes of a cumulative risk assessment as a part of the tolerance reassessment process for Acetochlor, Alachlor, and Butachlor, these three pesticides will be considered as a Common Mechanism Group. Following the initiation of a cumulative risk assessment, further analyses of new or existing data may occur which could impact the Agency's evaluation of specific members of this group or the group as a whole.

cc: Steven L. Johnson, OPPTS, Assistant Administrator
Other OPP Division Directors and Associate Division Directors

Attachments:

A) *Guidance for Identifying Pesticide Chemicals and Other Substances that Have a Common Mechanism of Toxicity,* Office of Pesticide Programs, USEPA (issued for public comment in August 1998; issued in revised form January 29, 1999).
B) *The Grouping of a Series of Chloroacetanilide Pesticides Based on a Common Mechanism of Toxicity*, Office of Pesticide Programs, USEPA (June 7, 2001).
C) SAP REPORT, April 28, 1997. Report of the FIFRA Scientific Advisory Panel

Meeting, March 19-20, 1997, held at the Crystal Gateway Marriott, 1700 Jefferson Davis Highway, Arlington, VA 22202.

D) Peer Review Self-Assessment and Verification Survey, Health Effects Division, Office of Pesticide Programs, USEPA (August 8, 1999).

Fax-On-Demand
Fax Number: (202) 401-0527
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GUIDANCE FOR IDENTIFYING PESTICIDE CHEMICALS AND OTHER SUBSTANCES THAT HAVE A COMMON MECHANISM OF TOXICITY

(January 29, 1999)

EXECUTIVE SUMMARY

The Food Quality Protection Act (FQPA) of 1996 requires the United States Environmental Protection Agency (EPA) to assess the cumulative risks to human health that can result from exposure to pesticides and other substances that are toxic by a common mechanism. The Agency is currently developing a process for performing cumulative risk assessments of this type. Such assessments will play an increasingly important role in the evaluation of risks posed by pesticides, and will improve the Agency's ability to make regulatory decisions that fully protect public health and sensitive subpopulations, including infants and children.

The identification of pesticides and other substances that cause a common toxic effect by a common mechanism is the first step of the cumulative risk assessment process. This document describes the approach that EPA will use for identifying pesticides and other substances that cause common toxic effects by common mechanisms of toxicity. Specifically, this document describes: EPA's interpretation of common mechanism of toxicity with respect to making a determination of safety; the specific steps that will be taken for identifying mechanisms of toxicity of pesticides and other substances that cause a common toxic effect; the types of data and their sources that are needed; how these data are to be used in reaching conclusions regarding commonality of mechanisms of toxicity; and criteria the Agency will use for categorizing pesticides and other substances for purposes of cumulative risk assessments. Details on the other aspects of the cumulative risk assessment process will be discussed in a separate document.

This document was developed from a draft version entitled *Guidance for Identifying Pesticide Chemicals that Have a Common Mechanism of Toxicity, for Use in Assessing the Cumulative Toxic Effects of Pesticides,* that was released for public comment in August of 1998 (FR 63 42031, FRL-5797-7). The Agency received comments from various organizations. Each of the commentors offered recommendations for improving the science policy. All comments were extensively evaluated and considered by the Agency. This revised version embodies many of the sentiments and recommendations of the commentors. The public comments, as well as a detailed summary of the Agency's response to the comments are being made available in the Federal Register.

I. INTRODUCTION

The Food Quality Protection Act (FQPA) of 1996 stipulates, among other things,[1] that when determining the safety of a pesticide chemical EPA shall base its assessment of the risk posed by the pesticide chemical on: aggregate (i.e., total dietary, residential, and other non-occupational) exposure to the pesticide and available information concerning the cumulative effects to human health that may result from dietary, residential, or other non-occupational exposure to other substances[2] that have a common mechanism of toxicity. The Act specifically mandates the Agency to consider the special susceptibility of infants and children to the toxic effects caused by pesticides. The Agency must also base its risk assessment on available information concerning the *cumulative* effects on infants and children to the pesticide and other substances that have a *common mechanism of toxicity*. The reason for consideration of these factors is due to the possibility that low-level exposures to multiple substances that cause a common toxic effect by a common mechanism could lead to the same adverse health effect as would a higher level of exposure to any of the chemicals individually. A person exposed to a pesticide at a level that is considered safe may in fact experience harm if that person is also exposed to other substances that cause a common toxic effect by a mechanism common with that of the subject pesticide, even if the individual exposure levels to the other substances are also considered safe.

Hence, in assessing the risks posed by a given pesticide chemical, EPA must assess the cumulative risks to human health that can result from exposure to the pesticide, as well as from other pesticide chemicals and other substances that are toxic by a common mechanism. The goal of a cumulative risk assessment, in regard to implementing FFDCA as amended by FQPA, is to characterize the potential for a cumulative toxic effect and the magnitude of the effect in individuals exposed to pesticides and other substances that cause a common toxic effect by a common mechanism. In order to assess these cumulative toxic effects, the Agency needs to first identify and categorize those pesticides and other substances that cause a common toxic effect by a common mechanism. **The purpose of this document is to describe the approach that EPA will use for identifying and categorizing pesticides and other substances that cause common toxic effects from common mechanisms of toxicity.** Specifically, this document describes:

- EPA's interpretation of common mechanism of toxicity with respect to making a determination of safety under FFDCA as amended by FQPA;

- The specific steps that need to be taken for identifying mechanisms of toxicity of pesticides and other substances that cause a common toxic effect;

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[1] For details see *The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and Federal Food, Drug, and Cosmetic Act (FFDCA) As Amended by the Food Quality Protection Act (FQPA) of August 3, 1996;* U.S. Environmental Protection Agency, Office of Pesticide Programs, document # 730L97001, March, 1997.

[2] "Other substances" includes pesticide chemicals, pharmaceutical substances (e.g., drug products), industrial chemicals, and other substances to which the general population is exposed.

- The types of data (and their sources) that are needed for doing so;

- How these data are to be used in reaching conclusions regarding commonality of mechanisms of toxicity;

- Criteria the Agency will use for categorizing pesticides and other substances for purposes of cumulative risk assessments.

This document does not address how EPA will assess cumulative toxicity when making determinations of safety. This topic will be discussed in a forthcoming Agency science policy document.

II. DEFINITIONS OF TERMS

This document uses a number of terms that are necessary for discussions of toxic effects, mechanism of toxicity, and the identification of substances that cause a common toxic effect by a common mechanism. These terms are not defined (some are not mentioned) in FQPA. The definitions presented here represent EPA's interpretation of the terms for purposes of implementing the requirements of FQPA.

Analog(s). Analog is a generic term used to describe substances that are chemically closely related. Structural analogs are substances that have similar or nearly identical molecular structures. Structural analogs may or may not have similar or identical biological properties.

Toxic Effect. A toxic effect is an effect known (or can reasonably be expected) to occur in humans, that results from exposure to a chemical substance and that will or can reasonably be expected to endanger or adversely affect quality of life. Some examples of toxic effects are acute lethality, loss of hearing, renal tubule necrosis and cardiomyopathy, to name just a few.[3]

Site of a Toxic Effect. The site of a toxic effect is the specific anatomical or physiological site or locus (e.g., organ or tissue) at which the effect occurs.

Common Toxic Effect. A pesticide and another substance that are known to cause the same toxic effect in or at the same anatomical or physiological site or locus (e.g., same organ or tissue) are said to cause a common toxic effect. Thus, a toxic effect observed in studies involving animals or humans exposed to a pesticide chemical is considered common with a toxic effect caused by another chemical if there is concordance with both site and nature of the effect.

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[3] Toxic effect is not synonymous with toxic endpoint. Toxic endpoint is a quantitative expression of a toxic effect occuring at a given level of exposure. For example, acute lethality is a toxic effect, whereas an LD_{50} value (median lethal dose) is the toxic endpoint that pertains to the effect.

Cumulative Toxic Effect. A cumulative toxic effect is the net change in magnitude of a common toxic effect resulting from exposure to two or more substances that cause the common toxic effect by a common mechanism, relative to the magnitude of the common toxic effect caused by exposure to any of the substances individually.

Toxophore. Substances that are capable of causing a toxic effect contain a structural feature or moiety that bestows the toxic property. This structural feature or moiety is referred to generically as the toxophore, or toxophoric moiety[4]. A toxic substance elicits its toxicity through interaction of its toxophore with a biomolecular site (e.g., receptor)[5] in cells of tissue or organs to cause changes or alterations in normal cellular biochemistry. These biochemical changes or alterations lead to disruption of the physiological process(es) the tissue or organs perform and, ultimately, the toxic effect. The toxicity of many substances, however, is not due to a direct interaction with a biomolecular site. Rather, the toxicity results from metabolism of a structural substituent to a toxophore, which then causes the toxicity. Metabolic pathways that lead to toxicity are often called **bioactivation pathways.**

Mechanism of Toxicity.[6] Mechanism of toxicity is defined as the major steps leading to a toxic effect following interaction of a pesticide with biological targets. All steps leading to an effect do not need to be specifically understood. Rather, it is the identification of the crucial events following chemical interaction that are required in order to describe a mechanism of toxicity. Generally, the more that is understood about the various steps in the pathway leading to an adverse effect, the more confident one is about the mechanism of toxicity. For instance, a mechanism of toxicity may be described by knowing the cascade of effects such as the following: a chemical binds to a given biological target *in vitro*, and causes the receptor-related molecular response; *in vivo* it also leads to the molecular response and causes a number of intervening biological and morphological steps that result in an adverse effect. Other processes may describe a mechanism of toxicity in other cases.

Common Mechanism of Toxicity. Common mechanism of toxicity pertains to two or more pesticide chemicals or other substances that cause a common toxic effect to human health by the same, or essentially the same, sequence of major biochemical events. Hence, the underlying basis of the toxicity is the same, or

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[4] The term “toxophore” with respect to toxic substances, is akin to the term “pharmacophore” with respect to drug substances: the pharmacophore is that structural moiety of a drug substance or substances which imparts a desired pharmacological property.

[5] A biomolecular site refers to a specific area on a particular type of biomolecule (e.g, DNA, RNA, peptide, protein, lipoprotein, enzyme, etc.) within a cell. The toxophoric portion of a given pesticide may interact reversibly or irreversibly with its biomolecular site, depending upon the reactive nature of the toxophore and the biomolecular site.

[6] In the context of this document, mechanism of toxicity refers to the mechanism by which a pesticide substance is toxic to humans or experimental animals, and not the mechanism by which it is toxic to target or intended species (i.e., its mechanism of pesticidal action). With some pesticides, however, the mechanism responsible for causing toxicity to humans or experimental animals is similar to the mechanism of pesticidal action.

essentially the same, for each chemical.

Toxic Action. Toxic action of a given substance is its interaction with biological targets, to lead to a toxic effect.

Site of Toxic Action. The site of toxic action of a given substance is the anatomical or physiological site(s), locus, or loci at which takes place the interaction of the substance with its biological targets, to lead to a toxic effect.

Structure-Activity Relationships. Substances that contain or are bioactivated to the same toxophore may cause a common toxic effect by a common mechanism. The relative toxic efficacy and potency[7] among the substances in their ability to cause the toxic effect may vary. Differences in potency or efficacy are directly related to: the specific or incremental structural differences between the substances; the influence these differences have on the ability of the toxophore to reach and interact with its biomolecular site of action; and on the intrinsic abilities of each of the substances to cause the effect. The ability of two or more structurally-related substances to cause a common toxic effect and the influence that their structural differences have on toxic efficacy and potency are referred to as structure-activity[8] relationships.

Weight-of-Evidence. Weight-of-evidence refers to a qualitative scientific evaluation of a chemical substance for a specific purpose. A weight-of-evidence evaluation involves a detailed analysis of several or more data elements, such as data from different toxicity tests, pharmacokinetic data, and chemistry data, followed by a conclusion in which a hypothesis is developed, or selected from previous hypotheses.

III. PROCESS FOR IDENTIFYING PESTICIDE CHEMICALS AND OTHER SUBSTANCES THAT HAVE A COMMON MECHANISM OF TOXICITY.

To assess the cumulative toxicity of pesticides and other substances that cause common toxic effects by common mechanisms, EPA will first need to identify those pesticides and other substances that cause common toxic effects by common mechanisms, and then group them in accordance with commonality of toxic effect and toxic mechanism. Once grouped, combined risk assessments can be performed and the potential for cumulative toxicity that may result from exposure to substances within a group can be characterized.

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[7] *Toxic efficacy* is the intrinsic ability for a substance to produce a given toxic effect. *Maximal toxic efficacy* is reached when an increase in dose no longer causes an increase in the magnitude (intensity) of the effect. *Toxic potency* is the magnitude of the toxic effect that results from a given exposure level (or dose), or the range in magnitude of the toxic effect that corresponds to a range in levels of exposure. *Relative toxic potency* refers to a comparison of the exposure level or dose required of an individual substance to the exposure levels or doses required of other substances to cause a common toxic effect of an equivalent magnitude (e.g, LD_{50}, ED_{50}) by a common mechanism of toxicity.

[8] In the context of this document the term “activity” is synonymous with toxicity.

The conceptual framework of the process that EPA will use to identify pesticide chemicals and other substances that cause a common toxic effect by a common mechanism is illustrated in Figure 1. This process is designed to enable EPA to make accurate identification and categorization of pesticides and other substances that are toxic from a common mechanism, in both a timely and resource-effective manner. (Specific examples of the application of this process are in preparation, and will be made available at a future date.) To implement the process, the Agency has convened a multidisciplinary team of EPA scientists who are experts in chemistry, biology, pharmacology, toxicology and pharmacokinetics. It is the responsibility of this team to identify and analyze data and information pertaining to toxic mechanisms, and to make expert judgements regarding mechanisms of toxicity of pesticides and other substances. The following policies and practices will be used by the Agency for identifying chemicals that have a common mechanism of toxicity:

- A thorough identification and analysis of all relevant information will be undertaken for each pesticide chemical and other substances under consideration. This will provide the basis for identifying underlying mechanisms of toxicity;

- A "weight-of-evidence" approach will be used to support the development of hypotheses pertaining to mechanisms of toxicity. Generally, no single piece of information will suffice to support the characterization of a specific or common mechanism of toxicity; this finding will be supported by the analysis and inter-relationships of available pieces of information;

- External review of EPA's decisions concerning: utilization of established toxic mechanisms; determination of toxic mechanisms for specific substances; and grouping of substances by mechanism of toxicity will be solicited as needed .

When identifying toxic effects, common toxic effects, mechanisms of toxicity, and common mechanisms of toxicity for purposes of grouping substances that cause a common toxic effect by a common mechanism of toxicity, care must be taken not to confuse "mechanism of toxicity" with "site of toxic action," or "site of toxic action" with "toxic effect" or "site of toxic effect." (These terms are defined near the beginning of this document.) With many substances, the site of a toxic effect is the same as the site of toxic action. It is also true, however, that with many other substances the site of a toxic effect may be different than site of toxic action. For example, a substance inhibits the catalytic activity of the peroxidase enzyme within the thyroid gland. Inhibition of this enzyme prevents the synthesis of thyroxine and triiodothyronine, and ultimately leads to hypothyroidism, the toxic effect. In this case, the site of the toxic effect is the same as the site of toxic action: the thyroid gland. Another substance known to cause hypothyroidism does so by preventing the synthesis of thyroid-stimulating hormone within the anterior pituitary gland. Here the site of the toxic effect is the thyroid gland, but the site of toxic action is the anterior pituitary gland. Although these two substances cause a common toxic effect, they would not be considered for cumulative risk assessment because they have different mechanisms of toxicity.

Many substances can cause more than one toxic effect, depending upon level of exposure, and do so by different mechanisms of toxicity that take place at different sites of toxic action. However, a chemical may also cause multiple toxic effects at multiple sites from a single mechanism of toxicity taking place at a single site of toxic action, provided that the function initially altered at the site of toxic action normally controls other functions at distant sites. For example, a substance that prevents the conversion of cholesterol to corticosteroid hormones in the adrenal cortex would ultimately cause many effects throughout the body that would differ in site and nature. The Agency will group substances that cause multiple toxic effects by a common mechanism from a common site of toxic action (e.g., the multiple effects caused by certain endocrine disruptors) for purposes of cumulative risk assessment, provided at least one of the toxic effects is common among the substances.

Step 1. Identify a Candidate Set of Substances That Might Cause a Common Toxic Effect by a Common Mechanism of Toxicity. The process of identifying pesticides and other substances that have a common mechanism of toxicity begins with a preliminary grouping of chemicals that might cause a common toxic effect by a common mechanism of toxicity (step 1, Figure 1). Substances that are related structurally, or have a similar mechanism of pesticidal action, or share a general mechanism of mammalian toxicity or cause what could be a common toxic effect in humans or experimental animals are those that could cause a common toxic effect by a common mechanism. Hence, the initial, preliminary grouping of substances will be based upon at least one of the following criteria:

- structural similarity;
- mechanism of pesticidal action;
- general mechanism of mammalian toxicity;
- a particular toxic effect.

Use of structural similarity as a starting point for grouping chemicals relies on the assumption that substances that are structurally analogous could contain a common toxophore (or may yield a common toxophore upon metabolism) and may interact analogously with cellular biomolecular sites to cause a common toxic effect. To identify pesticides and other substances that are structurally similar, the Agency will perform substructure searches in databases containing: registered pesticides; pesticides for which there are import tolerances; and other substances (e.g., pharmaceuticals, industrial chemicals) that are used in commerce in the United States. Search queries for identification of structurally similar substances may include, for example: toxophore (if known) or metabolic precursor of the toxophore; base structure; and accompanying functional groups or other substituents that may impact on the propensity of a substance to produce a toxicological response common with those of structurally-related chemicals.

Preliminary grouping of pesticides based on mechanism of pesticidal action is justifiable because the mechanisms by which a number of pesticides are toxic to humans are fundamentally similar or, in some cases, identical to their mechanisms of intended toxicity to pests. With such pesticides the portion of the molecule that is responsible for pesticidal action is also responsible for human toxicity (i.e., the portion of the molecule that bestows pesticidal activity is also the human toxophore). The pesticidal action and human toxicity of these pesticides are often due to analogous interactions of their toxophores with specific biomolecular sites that are common to pests and humans, respectively.

Preliminary grouping of pesticides and other substances that share a general mechanism of mammalian toxicity is based on the possibility that such substances may cause a common toxic effect. Examples of general mechanism of toxicity include, for example, substances that uncouple oxidative phosphorylation, or substances that are known to undergo the same or similar bioactivation pathways, or that are metabolized to the same or analogous metabolites that are toxic.

Preliminary grouping of pesticides and other substances that cause a particular toxic effect known to occur in experimental animals or humans is based on the possibilty that the effect could be common (i.e., concordant in both site and nature), and that commonality in toxicity among two or more substances could be due to a common mechanism. Since this type of grouping is functionally-based, not structure-based, it enables the identification of structurally unrelated substances that cause a common toxic effect from a common mechanism that otherwise may not be identifiable from groupings based on structural similarity or mode of pesticidal action alone.

Not all toxic effects can be used as a preliminary basis for grouping substances. Toxic effects which have many possible unrelated causes, or which could be defined as nonspecific in origin are not appropriate as the primary basis for initial grouping of chemicals. These effects, such as body weight changes or death, can result from many unrelated factors and are usually of limited value in understanding mechanism of toxicity. Therefore, such generalized effects, which could have many different causes, ordinarily will not be used as a basis for initial grouping of pesticides. An exception, however, is genetic alterations. While genetic alterations can result from a variety of causes, knowledge of the mechanism by which a chemical substance causes genetic alterations can provide insight into the mechanism by which it causes adverse human health effects. Therefore, data for chemicals with common mutagenic effects may serve as a basis for initial grouping of such chemicals.

Following preliminary grouping of substances using any of the criteria described above, other substances that are mammalian metabolic precursors to the substances identified under step 1 will be added to the initial grouping. The basis for including a metabolic precursor to a substance identified under step 1 is that since it is metabolized to the substance, it may cause a common toxic effect by a mechanism common with that of the substance.

It is important to emphasize and to make clear that the purpose of step 1 is for *preliminary grouping only*, and that substances (including any metabolic precursors) identified under this step will not be included in a cumulative risk assessment if it is determined that they do not cause a common toxic effect by a common mechanism. For example, while some substances that contain the same toxophore, or that are otherwise structurally analogous, may cause a common toxic effect by a common mechanism, others may not. It is also possible for substances to cause a particular toxic effect in which the nature of the toxic effect caused by each substance is the same, but the organ or specific location in the body where the effect occurs differs among the substances. It is also possible for two substances, even those that are structurally analogous, to cause entirely different toxic effects. Such differences between location or nature of a toxic effect can be ascribed to the specific structural and physicochemical differences between the substances, and the effect these differences have on their respective pharmacokinetics (i.e., absorption, distribution, metabolism, and excretion of each substance) or pharmacodynamics (i.e., the interaction of the toxophore with biomolecular sites). In these instances the policy of the Agency is *not* to group such substances for cumulative risk assessment purposes, because the toxic effects are not common, as defined earlier. It is also possible with substances that cause a common toxic effect to cause the effect by different mechanisms of toxicity. Conversely, substances that share a general mechanism of toxicity may not necessarily cause a common toxic effect(s). Furthermore, substances that have a common mechanism of pesticidal action may not necessarily have a common mechanism of toxicity in mammals. Again, in these instances the policy of the Agency is not to group such substances for cumulative risk assessment purposes because they are not consistent with the definition of common mechanism of toxicity.

It must also be stressed that step 1 (like step 2 discussed below) is a very inclusive and necessary screening step to identify preliminary groupings of substances for a rigorous assessment. In particular, the criteria used in step 1 are very broad, and thus there is a real possibility that a substantial portion of the pesticide chemicals and other substances which are included in a preliminary grouping may not have a common mechanism of toxicity, and many will be dropped from a group in subsequent steps. Accordingly, EPA does not regard information which shows substances meet the step 1 (and step 2) criteria for grouping as reliable by itself to conclude that such substances have a common mechanism of toxicity. Nor does such information create a sufficient presumption of the existence of a common mechanism of toxicity that it compels EPA to complete the remaining steps described below before making its safety determination.

Hence, only those substances that EPA determines, through the in-depth review described below, cause a common toxic effect by a common mechanism will be considered for cumulative risk assessment. As shown in the Figure, this examination will involve: a thorough evaluation of toxicity data to determine which substances identified under step 1 cause a common toxic effect; determination of the mechanism of toxicity by which each substance causes the common toxic effect; and subsequent comparison of each mechanism to confirm or rule-out commonality. It is likely that EPA will conclude a substantial portion of the substances identified in step 1 should not be included in a cumulative risk assessment.

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

Step 2. Definitively Identify Those Substances from Step 1 That Cause a Common Effect. The primary purpose of step 2 is to further refine the preliminary grouping created at step 1 by screening out substances that obviously do not cause a *common toxic effect.* Following the preliminary grouping of substances (step 1, Figure 1) a detailed evaluation of available toxicology data for each substance will be undertaken to identify and characterize the toxic effects caused by each substance, and to determine which of the substances cause toxic effects that are common with other substances (i.e., toxic effects that are concordant in both site and nature). A primary data set to be used by EPA will be toxicity data generated in support of regulatory activities as outlined in 40 CFR 158. The Agency may also use toxicity data obtained from other studies, such as those described in government reports, or the published literature. The evaluation of toxicology data for purposes of identifying and characterizing toxic effects will be conducted in a manner similar to that used by EPA in its pesticide registration and re-registration programs.

Most substances, depending upon level of exposure, can elicit more than one toxic effect (albeit one toxic effect is generally more readily elicitable than the others). All toxic effects caused by each substance, regardless of the exposure levels required to induce the effects, will be evaluated and compared to the toxic effects caused by the other substances. Only those substances that cause a common toxic effect will remain grouped. Thus, for those substances initially grouped (step 1) using the "particular toxic effect" criterion (step 1), a determination as to which substances the toxic effect is in fact common will need to be made. The toxicity data for substances initially grouped using any of the other criteria in step 1 will also be evaluated to determine which of these substances cause a common toxic effect. Substances may be placed in more than one group in instances where substances cause more than one common toxic effect. Pesticide chemicals that do not cause a toxic effect that is common with at least one other substance identified under step 1 will be eliminated from the group and, thus, will not undergo further cumulative risk consideration.

Step 3. Determine the Toxic Mechanism(s) by Which Each Substance Causes a Common Toxic Effect. The next phase of the review process (step 3, Figure 1) is to determine the mechanisms by which the substances cause the common toxic effect(s) identified under step 2 (Figure 1). Generally, the more that is understood about the various biochemical events that lead to a toxic effect, the more apparent and scientifically acceptable is the mechanism of toxicity. While desirable, all of the specific biochemical events involving a substance in the causation of its toxicity do not need to be known or completely characterized in order to describe its mechanism of toxicity. What is needed, as a minimum, is an understanding of those biochemical events that are most crucial in causing the toxicity. Once the critical biochemical events pertaining to toxicity are understood for each substance, they can be compared and identification of those substances that are toxic from a common mechanism can be made. Hence, the goal of step 3 is to determine, to the extent possible for each substance identified under step 2 as causing a common toxic effect, those biochemical events that are most critical in causing the effect.

The toxic mechanisms of some classes of substances in causing a given toxic effect have been characterized, and are described in various literature sources (e.g., textbooks, journals, etc.). These mechanisms were elucidated from the development and comprehensive analysis of data pertaining to the structure, pharmacokinetics and toxicity of the substances and their analogs. The toxophoric moieties and structure-activity relationships of many of these chemical classes were similarly characterized. The toxic mechanisms, toxophores and structure-activity relationships of other pesticides, however, have not been fully characterized, either because of insufficient data, or because available data have not yet been fully analyzed.

Rather than reexamining *de novo* all of the relevant data, EPA will assume that a substance is toxic by the mechanism that has been previously determined provided that the mechanism is consistent with current toxicological theory and deemed scientifically plausible by the Agency for these purposes. Thus, identification of toxic mechanisms will involve an initial search of Agency databases and the literature (step 3a, Figure 1) for assessments or studies that describe mechanisms of toxicity for any of the pesticides grouped in step 2. The types of literature sources that will be searched and used include standard reference and text books, peer-reviewed journals, government reports, and study reports submitted to the Agency. This will allow segregation of the substances into two sub-groups: those for which mechanism(s) of causing a common toxic effect have been determined; and those for which their mechanism(s) have not been determined. When deemed necessary, more comprehensive literature or Agency database searches will need to be conducted to identify data that support or invalidate previously determined mechanisms of toxicity for which uncertainty exists.

EPA will attempt to determine the mechanisms of toxicity of those substances whose toxic mechanisms are not known or not well understood, or for which there is an absence of direct mechanistic data. The determination of a toxic mechanism will be based upon an evaluation of various data elements. The types of data and information that the Agency will use to develop a scientifically defensible determination of a given pesticide's toxic mechanism are structural data, pharmacokinetic data, and toxicity data. In situations in which such data are not available or are insufficient for a pesticide, the Agency will review and may use mechanistic, structural, pharmacokinetic or toxicity data pertaining to one or more analogs of the pesticide (or other substance) as a basis for determining the toxic mechanism of the pesticide. Identifying and obtaining pesticide or analog data will involve a comprehensive search of the literature and Agency databases. A primary source of these data and information will be studies that have been submitted to the Agency in support of registration and reregistration decisions. Other sources of data will include peer-reviewed journals, text books and government reports.

The Agency will analyze these data and, using a weight-of-evidence approach, will attempt to determine the major biochemical events involving a pesticide (or other substance) that are most critical in causing its toxicity (step 3b). From an analysis of a substance's structure, for example, the recognition of moieties that are known or expected to react with biological macromolecules, or are known or expected to be metabolized to reactive (e.g., radical, electrophilic) intermediates, or are otherwise known or expected to bestow toxicity may allow one to infer one or more biochemical events that are responsible for the substance's toxicity. Data that define the metabolism, distribution and excretion of a pesticide in the body are also very useful for determining its mechanism of toxicity. Metabolism data that show the formation of toxic metabolites *in vivo* are especially useful for characterizing metabolic pathways which may be operative in causing toxic effects. Distribution and excretion data show the partitioning patterns of a substance in the body, and may in some cases be used to infer the types of metabolic transformations that are most likely to occur and where they are most likely to take place. These data, in conjunction with structural and toxicity data, may also provide explanations for differences in toxicity of structurally similar substances. Toxicity data can be helpful in the determination of a toxic mechanism in many ways. Genetic alterations, for example, are important in the causation of cancers and developmental effects. Tests for genetic alterations that show that a substance (or a metabolite thereof) forms a covalent adduct with DNA may be useful to infer or support a mechanism by which a pesticide known to cause cancer or developmental toxicity causes either of these effects.

Data pertaining to analogs of a pesticide or other substance will be reviewed and may be used in situations in which mechanism-related data are not available for the pesticide. An established mechanism of toxicity of a pesticide's analog(s), for example, may serve as a basis for determining the toxic mechanism of the pesticide. Conclusions based on the toxic mechanisms of an analog or analogs will only be made when: there is evidence that shows that the toxicological effects caused by the pesticide and the analogs are common; there is sufficient evidence that supports the toxic mechanism of the analog(s); and there is sufficient evidence for the Agency to conclude that the mechanism of toxicity of the pesticide is common with the mechanism of toxicity of the analog(s). Pharmacokinetic, toxicity and structure-activity relationship data that are available for analogs of a pesticide will also be used as a basis for determining the toxic mechanism of the pesticide (step 3b, Figure 1). For example, it is stated above that test data pertaining to genetic alterations may be useful to infer or support a mechanism of a pesticide known to cause cancer or developmental toxicity. Genetic alterations data available for analogs of a pesticide known to cause cancer or developmental toxicity may be useful for inferring the mechanism by which the pesticide causes these effects. Genetic alterations that are similar among a pesticide and its analogs are also useful in a weight-of-evidence confirmation of the validity of such inferences, particularly when mechanistic data are available for the analog but not for the subject pesticide.

Relationships between structure and toxicity within a given series of structurally-similar substances or of a single given substance are often discernable from an analysis of: the general structure; the chemical properties of the substance(s); information pertaining to the pharmacokinetics and toxicity of the substance(s); and the structural differences within the series and their corresponding affect on toxic efficacy and potency. While knowledge of the mechanism of toxicity is usually not necessary in order to discern a causal relationship between structure and activity (toxicity), the relationship becomes more apparent and more useful when the mechanism of toxicity is known. Once deduced, the structure-activity relationship of the substance or the series can be useful for inferring the likelihood of an analogous, untested chemical to cause the same toxicological effect, and for estimating its toxic potency. In cases where the mechanism of toxicity is known for a substance or a group of substances, structure-activity relationships are useful for inferring the mechanism of toxicity of an analogous, untested substance and for supporting or refuting proposed mechanisms of toxicity of analogous untested substances.

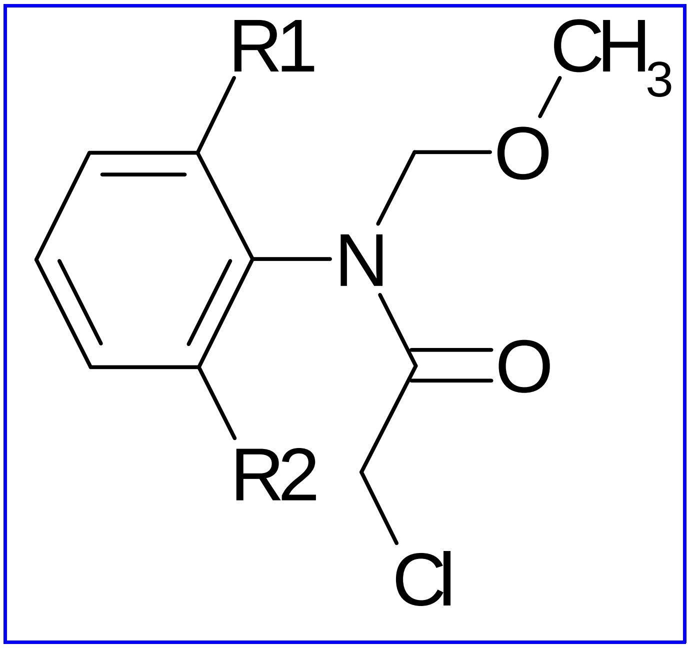
Steps 4 and 5. Comparison of Mechanisms of Toxicity (Step 4) and Refined Grouping of Substances (Step 5). Once the mechanism of toxicity of each substance has been identified, comparisons of mechanisms will be made to determine which substances identified under step 2 as causing a given common toxic effect do so by a common mechanism. Determinations that two or more substances are toxic by a common mechanism will be based on similarities in both the nature and sequence of the major biochemical events that cause toxicity. Mechanistic similarities that would support a finding of a common toxic mechanism include, for example, analogous interactions of the pesticides or other substances with identical or similar biological targets, or the occurence of similar metabolic transformations that yield common or structurally analogous metabolites that interact with similar biological targets, or that are otherwise involved in causing toxicity. Substances that cause a common toxic effect by different mechanisms will excluded from the refined grouping (Step 5).

Peer review of EPA's decisions concerning: utilization of established toxic mechanisms; identification of toxic mechanisms for specific substances; and grouping (or non-grouping) of substances for purposes of cumulative risk assessment will be solicited in situations in which the Agency believes additional evaluation is needed to ensure that Agency decisions are consistent, well-reasoned and reflect current scientific thinking.

IV. ASSESSING THE CUMULATIVE TOXICITY POSED BY TWO OR MORE SUBSTANCES THAT ARE TOXIC BY A COMMON MECHANISM.

Cumulative toxicity represents the net change in toxicity resulting from exposure to two or more chemical substances, relative to the toxicity caused by each substance alone. The evaluation of cumulative toxicity will be conducted in accordance to a cumulative risk assessment process being developed by the Agency. The goal of the cumulative risk assessment process, in regard to implementing FFDCA as amended by FQPA, is to characterize the potential for a cumulative toxic effect and the magnitude of the effect in individuals following known or anticipated exposures to substances that cause the effect by a common mechanism. Pesticide chemicals and other substances within a refined common mechanism grouping (step 5, Figure 1) will undergo cumulative risk assessment to determine the potential cumulative toxicity posed by exposures to such substances. This will involve consideration of a number of factors that pertain to: exposure; the pharmacokinetics of each substance; the nature of the common toxic effect; the pharmacodynamics of each substance in causing the effect; pharmacokinetic or pharmacodynamic interactions that may take place between the substances; subpopulations for which exposures are anticipated; and susceptibility and sensitivity of exposed individuals or subpopulations to the common toxic effect. A discussion of how these factors affect cumulative toxicity is beyond the scope of this document. In addition to the substances wihitn a refined grouping, the Agency will also consider the potential contribution to cumulative toxicity from other substances that undergo environmental degradation or metabolism in plants to any of the substances within the refined group. Substances that degrade in the environment, or that are metabolized in plants to substances in the refined grouping will be included in a cumulative risk assessment because such precursor substances may represent an additional source of exposure to the substances in the refined grouping. The cumulative risk assessment process that the Agency will use will be described in a forthcoming Agency science policy guidance document. The document will include detailed discussions of the above factors, how these and other factors will be considered by the Agency in assessing cumulative toxicity, and what the Agency will do when there are data gaps with the above factors.

The Grouping of a Series of Chloroacetanilide Pesticides Based on a Common Mechanism of Toxicity



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Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
Washington D.C. 20460

June 7, 2001

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Executive Summary

This document discusses the available scientific evidence for determining whether a common mechanism of toxicity exists among certain chloroacetanilide pesticides. The weight-of-the-evidence (WOE) analysis used is similar to the general approach outlined in the January 29, 1999 ***Guidance for Identifying Pesticide Chemicals and Other Substances That Have A Common Mechanism of Toxicity*** [http//www.epa.gov/fedrgstr/EPA-PEST/1999/February/Day-05/6055.pdf]. The group of chloroacetanilide pesticides covered in this document consists of the following: **Acetochlor**, **Alachlor**, **Butachlor**, **Metolachlor** and **Propachlor**.

Treatment of laboratory animals with these chloroacetanilides results in toxic effects such as nasal turbinate tumors, thyroid follicular cell tumors, and stomach- and liver tumors. Based on the available evidence, only **Acetochlor**, **Alachlor**, and **Butachlor** can be grouped by common mechanisms of toxicity for nasal turbinate tumors. The mechanism for nasal turbinate tumors is postulated to be associated with cytotoxicity to the nasal olfactory epithelium, followed by regenerative cell proliferation and neoplasia.

Although the available evidence also supports grouping **Acetochlor**, **Alachlor**, and **Butachlor** by a common mechanism of toxicity based on thyroid follicular cell tumors, this endpoint is not considered appropriate for risk assessment, because the toxic effects were noted at doses above the Maximum Tolerated Dose (MTD). At this time there are inadequate data available for grouping chloroacetanilides based on stomach or liver tumors.

Thus, in the absence of additional evidence that may support an alternative grouping, the weight-of-evidence (WOE) supports grouping of **Acetochlor**, **Alachlor**, and **Butachlor** based on a common mechanism of toxicity for rat nasal turbinate tumors for purposes of a cumulative risk asssessment.

The present groupings were presented to the FIFRA Scientific Advisory Panel (SAP)as a draft on March 19, 1997. The SAP agreed with the Agency's conclusion that there is sufficient evidence to support the proposed grouping for the nasal turbinate tumors. The SAP recommended that regarding the thyroid tumors, even though the case study illustrated that a common mechanism could be used to group chloroacetanilides for the development of thyroid tumors, this endpoint should not be used because the toxic effects were seen at doses exceeding the Maximum Tolerated Dose (MTD).

The Grouping of a Series of Chloroacetanilide Pesticides Based on a Common Mechanism of Toxicity

I. Introduction

A. Background

The Food Quality Protection Act of 1996 (FQPA) requires EPA to consider “available information concerning the cumulative effects of [pesticide] residues and other substances that have a common mechanism of toxicity.” Sec. 408(b)(2)(D)(v) of the Federal Food Drug and Cosmetic Act. FQPA directs the Agency to consider “available information concerning the cumulative effects of such residues and other substances that have a common mechanism of toxicity.” Central to performing this task is the process of identification of those pesticide chemicals that can be grouped based on a common mechanism of toxicity.

In March of 1997 (FR 62 4283), the Agency presented to its FIFRA Scientific Advisory Panel (SAP) a draft “Guidance for Establishing a Common Mechanism of Toxicity for Use in Combined Risk Assessments”. In its recommendations [SAP Report dated April 28, 1997], the SAP supported the draft Guidance that the Agency had outlined for determining common mechanism of toxicity and endorsed the proposed weight of evidence approach for determining presence or absence of a common mechanism of toxicity.

At the same March 1997 meeting, the Agency also presented to the SAP a draft case study illustrating the application of the proposed Guidance to the grouping of a series of chloroacetanilide pesticides based on a common mechanism of toxicity. In its recommendations [SAP Report dated April 28, 1997] the SAP noted that the case study was ”.. excellent, well-presented, and very appropriate”. The SAP agreed with the Agency’s conclusion that there is sufficient evidence to support the grouping of certain chloroacetanilides that cause nasal turbinate tumors by a common mechanism of toxicity. Additionally, the SAP recommended that regarding the thyroid tumors, even though the case study illustrated certain chloroacetanilides had the capacity to cause the development of thyroid follicular cell tumors, this endpoint should not be used to support a finding that the chemicals shared a common mechanism of toxicity because the toxic effects were seen at doses exceeding the Maximum Tolerated Dose.

Subsequently, a draft version of the guidance entitled *Guidance for Identifying Pesticide Chemicals that Have a Common Mechanism of Toxicity*, for Use in Assessing the Cumulative Toxic Effects of Pesticides was released for public comment in August of 1998 (FR 63 42031, FRL-5797-7).

After extensive evaluation and consideration of comments received from the SAP and from various organizations, a revised document [referred to as the Guidance] was prepared. This revised document is entitled *Guidance for Identifying Pesticide Chemicals and Other Substances That Have A Common Mechanism of Toxicity* was released January 29, 1999 [http//www.epa.gov/fedrgstr/EPA-PEST/1999/February/Day-05/6055.pdf **or** Document No. 6055, Fax-on-Demand, (202)401-0527].

B Purpose

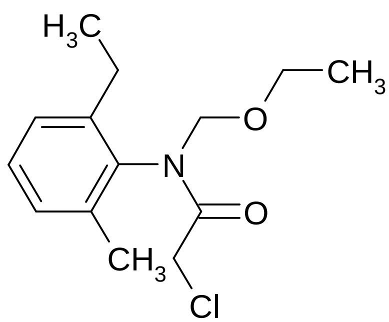
The purpose of this document is to evaluate whether the chloracetanilide pesticides share a common mechanism of toxicity taking into account the Guidance. This document incorporates additional information received by the Agency from Registrants under FIFRA or presented in the open literature since the 1997 document. Data submitted under FIFRA testing guidelines includes extensive standard toxicological information. Therefore, OPP has considered the new information submitted since 1997 and this new information reinforces its earlier conclusions.

OPP has used a weight-of-evidence (WOE) approach that considers all pertinent information to determine whether chemicals act via a common mechanism of toxicity. A stepwise process is outlined in the 1999 Guidance document that starts with an initial grouping of chemicals based on having shared structural, toxicological and/or pesticidal properties. In a second phase, the steps that define the mechanism of toxicity for one or more chemicals in the group is identified. Finally, structural, toxicological and pharmacokinetic/pharmacodynamic data for the remaining chemicals in the group are examined to determine by WOE which of these possess the same mode of toxic action as the other compound(s) in the group. All those chemicals found to share the same mode of action for a common toxic effect are considered to have been grouped by a common mechanism of toxicity.

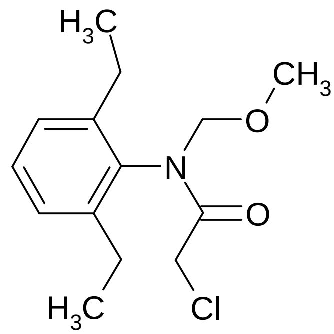
It should be noted that “mechanism of toxicity” is defined in the Guidance document (USEPA,1999) as “the major steps leading to an adverse health effect following an interaction of a pesticide with biological targets. All steps leading to an effect do not need to be specifically understood. Rather, it is the identification of the crucial events following chemical interaction that are required in order to describe a mechanism of toxicity.”

II. The Candidate Group of Pesticides

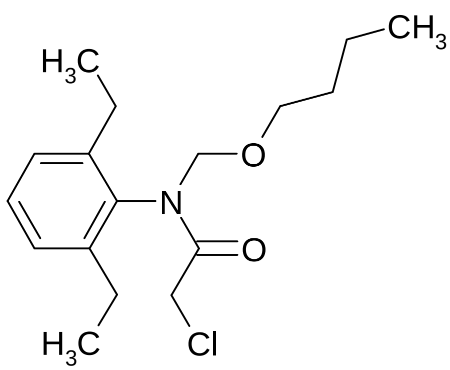
The compounds shown in Figure 1 are the **chloroacetanilide** pesticides considered for grouping via a common mechanism of toxicity. This group, hereafter referred to as the candidate group, initially was selected based upon them all possessing the **chloroacetanilide moiety**. It should be noted that diethatyl ethyl (Figure 2),although containing the chloroacetanilide moiety, will rapidly form a carboxylic acid, with a disposition and fate that is likely to be different from that of the other chloroacetanilides; and dimethanamid is not a chloroacetanilide. Although these two compounds appeared in the 1997 case study, they lack the structural and biochemical features to belong to the candidate group. Therefore, diethatyl ethyl and dimethanamid are not covered in this document.



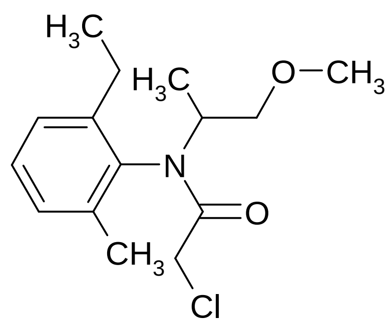
Acetochlor



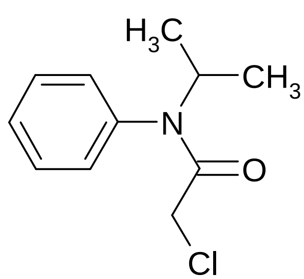
Alachlor



Butachlor



Metolachlor



Propachlor

Figure 1. Structures of the Chloroacetanilides in the Candidate Group.

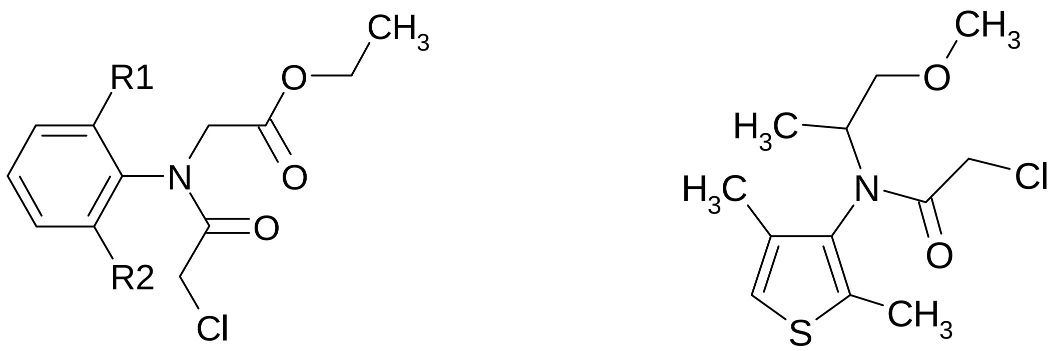


Figure 2. Structure of diethatyl ethyl (left) and dimethanamid (right)

III. Lines of Evidence

In this section, the various available lines of evidence to be used in weight-of-evidence evaluation for the compounds under consideration will be presented.

A. Structure Activity Considerations

In general, based on structure-activity relationships (SAR), the pesticides in a given mixture may be grouped according to their likelihood to generate a common type of reactive intermediate or their ability to mimic a common biologically active molecule that interferes with the normal homeostasis of the cell (e.g., via receptor binding, enzyme induction, etc.).

For the candidate group of chloroacetanilides, at least four reactive intermediates capable of eliciting toxic action may be conjectured. These include: (a) the active chlorine α to the carbonyl group, (b) a reactive benzoquinone imine intermediate, (c) formaldehyde, (d) an α,β-unsaturated aldehyde.

All 5 chloroacetanilides contain the direct-acting active chlorine in the α-position relative to the carbonyl group. This type of electrophilic reactive compound is expected to preferentially react with "soft" nucleophiles such as glutathione (GSH) and SH-containing proteins. All 5 chloroacetanilides are therefore expected to react with GSH to cause depletion of the protective nucleophile. Such depletion is expected to be of particular concern for tissues with relatively low level of endogenous GSH (e.g., blood, nasal tissue, stomach) rendering them more susceptible to the toxic action of this or other types of reactive intermediates. Alternatively, these pesticides may directly react with SH-containing proteins at or near the port of entry, or initial site of absorption (e.g., blood) to exert toxic action.

All chloroacetanilides in the candidate group are *potential* substrates for generating the reactive benzoquinone imine intermediate after N-dealkylation (by mixed function oxidase) and N-deacylation (by aryl amidase) of the pesticides with subsequent ring hydroxylation and oxidation. However, since N-dealkylation requires α-hydroxylation, **Metolachlor** and **Propachlor** are expected to be substantially poorer substrates because of steric hindrance at the α-carbon. Thus, only Acetochlor, Alachlor and Butachlor are, *a priori*, expected to have relatively good substrates for generating a benzoquinone imine intermediate. The benzoquinone imine intermediate, once formed, is capable of reacting with nucleophilic portions in macromolecules, particularly in tissues in which endogenous GSH is depleted.

Two pesticides in the candidate group -- **Alachlor** and **Metolachlor** – are potential generators of formaldehyde. Metabolic O-dealkylation of the terminal alkyl group is expected to yield the unstable N-methylol moiety which can spontaneously decompose to yield formaldehyde. Formaldehyde, once formed, may be rapidly detoxified or serve as cross-linking agent to initiate toxic action at or near the site of generation. Exposure to high doses of formaldehyde has been associated with the induction of nasal tumors. *In-situ* metabolic production of formaldehyde has been postulated to be the most likely reactive intermediate in the nasal carcinogenic action of hexamethylphosphoramide (HMPA).

Thus, based on SAR consideration of common reactive intermediates, only **Acetochlor**, **Alachlor** and **Butachlor** appear to share the potential for sharing a common mechanism of action in several aspects.

Much less is known about the ability of the chloroacetanilides in the candidate group to mimic a common biologically active molecule to interfere with the normal homeostasis of the cell to exert toxic action. There is evidence that several pesticides in this group are capable of inducing microsomal enzymes. However, there is no clear evidence to relate any structural moieties or features to their ability to bring about microsomal enzyme induction. There is some evidence that **Alachlor** and **Butachlor** may act as promoters in stomach carcinogenesis. There is no evidence, however, that they have any resemblance to the H_2 histamine antagonist and the proton pump inhibitor types of pharmaceutical stomach carcinogens.

B. Toxicological Considerations

Table 1 summarizes the toxic effects observed in chronic studies with the candidate chloroacetanilides. Effects common to three or more chemicals were seen in nasal tissue, thyroid, stomach, kidneys, and liver.

1. Nasal Tissue

Statistically significant increases in nasal tumors have been reported for **Acetochlor**, **Alachlor** and **Butachlor** in rats (Table 1). Additionally, nasal tumors (1 adenocarcinoma and 1 fibrosarcoma) have been reported in rats fed **Propachlor** at 3000 ppm in the diet. Although the results for propachlor did not reach statistical significance, nasal turbinate tumors are considered to be rare and these results are suggestive of a neoplastic response at that site. Additionally, the FIFRA SAP (meeting of September 18, 1991), indicated concern for the biological significance of the nasal tumors in rats fed **Metolachlor**. No nasal tumors have been reported for any of these chemicals for mice.

2. Thyroid Gland

Thyroid follicular cell tumors have been reported for **Acetochlor**, **Alachlor**, and **Butachlor** in rats (Table 1). Although **Propachlor** produced thyroid tumors in rats, these thyroid tumors were identified as C-cell tumors, not follicular cell tumors, as those seen for **Acetochlor**, **Alachlor**, and **Butachlor**. Thyroid tumors have not been reported for other chloroacetanilides in Table 1.

3. Stomach

Stomach tumors have been reported for **Acetochlo**r, **Alachlor** and **Butachlor** in rats (Table 1). Stomach lesions have been reported in CD-1 mice of both sexes administered **Propachlor** in the diet for 18 months at levels of 0, 100, 500, 1500 or 6000 ppm. Herniated mucosal glands into the submucosa/tunica muscularis were observed in both sexes at the highest dose and in some males at the next highest dose level. Males at the highest dose level also showed erosion/ulceration of the glandular mucosa of the stomach. No stomach tumors were reported for **Metolachlor**.

4. Kidney

Kidney effects have been reported for **Acetochlor**, **Alachlor** and **Butachlor**. **Acetochlor** showed histopathology in a 1-year dog study, increased relative kidney weights in a 2-year rat study and in a 78-week mouse study. **Alachlor** has shown increased kidney sclerosis in a 2-year rat study. **Butachlor** has shown chronic nephropathy in the rat in a 2-year study, in addition to kidney tumors.

5. Liver

Liver effects have been reported for most chemicals of the candidate series. **Acetochlor**, **Alachlor**, **Butachlor**, and **Propachlor** have produced increases in relative liver weights, coupled in some cases with hepatocellular hypertrophy. These changes are not inconsistent with an induction of microsomal enzymes. In fact, experimental data exist to indicate that **Acetochlor**, **Alachlor**, and **Butachlor** induce microsomal enzymes. Alachlor induces in rats hepatic T4-UDPGT, a microsomal enzyme, by 168-194% of control levels after 14 and 28 days of administration. The same enzyme is induced in rats by **Acetochlor** to 142-218% of controls by 14 days of treatment and by **Butachlor** to 166% of controls at 20 months of dietary administration. Additionally, **Propachlor** has been found to decrease the sleeping time in rats, suggestive of microsomal enzyme induction.

Liver tumors, summarized in Table 1, were observed in mice or rats for **Acetochlor**, **Metolachlor** and **Propachlor** in chronic studies. Metolachlor produced statistically significant increases in liver adenomas and combined adenomas/carcinomas in female rats and a statistically significant trend for liver tumors in male rats. **Propachlor** produced a statistically significant increase in hepatocellular tumors [adenomas, carcinomas, hepatoblastomas] in high dose male CD-1 mice.

Table 1. Toxicology Data for the Candidate Group of Chloroacetanilide Pesticides

Effects	Acetochlor	Alachlor	Butachlor
Nervous System	1-year dog: brain histopathology (50 mg/kg/d), salivation and neurotoxic signs (10 mg/kg/d).	2-Year rat: Compression atrophy of the brain 14 mg/kg/day	-
Renal System	1-Year dog: kidney histopathology (40 mg/kg/day). 2-Year rat (SD): kidney rel. wts. ↑ (79.6 mg/kg/d) 78- Week mouse (CD-1): absolute and relative kidney (↑, dose-related) tubular basophilia (↑).	2-Year rat: tubular sclerosis kidney ↑ (15 mg/kg/d) 18-Month Mouse: slight increases in tubular epithelium hyperplasia/regeneration in males.	2-Year rat (S-D): Chronic nephropathy increased (5 mg/kg/d). Kidney cortical tumors at 150 mg/kg/d). 2-Year rat (Fisher-344): Tubular cell hyperplasia & pelvic epithelial hyperplasia (40 mg/kg/d). No tumors.
Hematology/Clinical Chemistry	1-Year dog: Cholesterol (↑), 50 mg/kg/d 2-Year mouse: RBC, Hct and Hb (↓) at greater than 500 ppm	1-Year dog: Hemolytic anemia (3 mg/kg/d) in males; Hemosiderosis in kidney, liver, and spleen of males only.	1-Year dog: Cholesterol (↑), 25 mg/kg/d. 90-Day rat: Mild anemia plus spleen hemisiderosis at 3000-5000 ppm
Ovary/Testes	1-Year dog: testes weights ↓, tubular de-generation, hypospermia (40 & 50 mg/kg/d)	2-Year rat: ovarian wt ↓ (15 mg/kg/d)	-
Eye	2-Year rat (SD): ocular lesions (79 mg/kg/d) 2-Year mouse: positive trend for retinal degeneration (1500 & 5000 ppm) 78-Week mouse (CD-1): increased (not significantly) lens vacuolation at >100 ppm	2-Year rat: ocular lesions (uveal degeneration, 14 mg/kg/d plus corneal opacity at 42 mg/kg/d). 2-Year rat: ocular lesions (uveal degeneration, 15 mg/kg/d)	2-Year rat (Fisher-344): cataract & retinal atrophy significantly increased in females vs controls at 40 mg/kg/d
Liver	Increased relative liver weights in dog, rat (SD) and mouse chronic studies. 78-Week mouse (CD-1): significant increase in combined hepatocytic adenomas plus carcinomas observed in males only at the high dose.	6-Month dog: increased relative weight, 15 mg/kg/d, fatty degeneration/biliary hyperplasia, 25 mg/kg. 1-Year dog: Significantly increased absolute and relative weights in males only; values in females were increased but not significantly. 18-Month mouse: Hepatocellular hypertrophy in males only.	1-Year dog: hepatocellular swelling and increased liver weight (25 mg/kg/d). 2-Year rat (Fisher-344): significantly increased incidence of hepatocellular swelling, acidophilic foci and mixed cell foci of alteration in males. Significantly increased trends and pairwise incidence for hepatocellular tumors in males not considered treatment related.
Stomach	2-Year rat (SD): basal cell tumors (limited to 1♂ and 1♀ at the high dose).	2-Year rat (Long-Evans, 14-126 mg/kg/day): significant increasing trends and significant pair-wise increases in malignant mixed gastric adenocarcinoma and / or mixed gastric tumors combined in both sexes at the high dose. No stomach tumors were seen in another chronic study at doses of 0.5-15 mg/kg/day in the same strain.	2-Year rat (S-D 4.5-139 mg/kg/day ♂ and 5.7-190 mg/kg/day ♀) : Tumors limited to females. Significant increasing trends and pairwise comparison for carcinomas, carcinosarcomas, and combined carcinosarcomas / leiomyosarcomas. Significant increasing trend for leiomyosarcomas.
Thyroid	2-Year rat (SD, 0.8-79.6 mg/kg/day): Combined follicular cell tumors, significant positive trend in both sexes and pairwise significant increases in adenomas and combined adenomas/carcinomas at the high dose in females only.	2-Year rat (Long-Evans, 14-126 mg/kg/day): significant increasing trends and significant pair-wise increases in thyroid follicular cell adenomas, carcinomas and combined adenomas / carcinomas in males and significant increasing trends in thyroid follicular cell adenomas and combined adenomas carcinomas in females at the high dose (126 mg/kg/day). No thyroid tumors were seen in another chronic study at doses of 0.5-15 mg/kg/day in the same strain..	2-Year rat (S-D): Significant increasing trend in follicular cell adenomas and adenomas and/or carcinomas combined in males and significant increasing trend in follicular cell adenomas, carcinomas and adenomas and/or carcinomas combined in females; plus pairwise increases in follicular cell adenomas and adenomas and/or carcinomas combined in both sexes.
Nasal Tissues	2-Year rat (S-D, 0.8-79.6 mg/kg/day): significant pairwise increases in nasal epithelium adenomas were seen for both sexes at the high-dose vs. controls ($p \leq 0.01$ for trend and pairwise comparisons). Carcinomas of the nasal epithelium, although not statistically significant, had an incidence of 3% (♂) and 2% (♀) at the high dose only vs 0% in controls. There were statistically significant trends in combined carcinoma/adenoma for both sexes and in pairwise combined carcinoma/adenoma for both sexes at the high dose.	2-Year rat (Long-Evans): Significant increasing trends and pair-wise increases in nasal respiratory epithelium adenomas and combined adenomas/carcinomas in both sexes were observed in two studies at 14-126 mg/kg/day and 0.5-15 mg/kg/day..	2-Year rat (S-D): In males: Significant increasing trends and pair-wise increases in nasal respiratory/olfactory epithelium adenomas and combined adenomas/carcinomas; in females: significant increasing trends in nasal respiratory / olfactory epithelium adenomas, carcinomas and combined adenomas / carcinomas plus pair-wise increases in nasal respiratory/olfactory epithelium adenomas, and combined adenomas/carcinomas.

Table 1.Toxicology Data for the Candidate Group of Chloroacetanilide Pesticides (Continued)

Effects	Metolachlor	Propachlor
Nervous System	No	No
Renal System	No	No
Hematology/Clinical Chemistry	No	No
Ovary/Testes	No	2-Year (S-D)rats: there was a significant positive trend for benign and combined benign / malignant tumors and a pairwise significant increase in combined benign / malignant tumors of the ovarian granulosa / theca cells in high dose rats.
Eye	No	No
Liver	2-year rat [CD (SD) BR):statistically significant increases in liver adenomas and combined adenomas/carcinomas in female rats. In male rats there was a statistically significant trend, but not pair-wise significance for liver tumors	18-Month (CD-1) Mice: Dose-related increases in relative liver weights and in hepatocellular hypertrophy in both sexes. Additionally there was necrosis of individual hepatocytes, eosinophilic foci, teleangiectasis. There was a statistically significant trend and pairwise increase in hepatocellular tumors [adenomas, carcinomas, combined adenomas / carcinomas] in high dose (847.3 mg/kg/day) males.
Stomach	No Tumors	18-Months (CD-1) mice: herniated mucosal glands, erosion ulceration of the glandular mucosa. 2-Year Fischer rats: A single carcinoma of the glandular stomach was seen in one male at the high dose (125 mg/kg/day). The tumor was attributed to treatment but it was of a different type as that observed for Chlors-2 and -3
Thyroid	No	104-Week (S-D) rat at doses up to 24 mg/kg/day: there was a significant positive trend for adenomas and combined adenomas / carcinomas of C-cells for both sexes and a statistically significant increase in the incidence of combined C-cell adenomas / carcinomas in high-dose males. The dose was not considered to be adequately high for testing.
Nasal Tissues	2-Year rat [CD(SD)BR): Nasal tumors were not statistically significantly elevated but 1 adenocarcinoma (nasal gland) and 1 neurofibrosarcoma (peripheral nerve) were seen in high-dose males vs 0 in controls. Polipoid adenomas of the respiratory epithelium were seen in controls (1) and high dose males (1) and in mid-dose females (1). A squamous papilloma was seen in high-dose females and none in controls. Dosing is considered to be marginally adequate for carcinogenicity assessment.	No

C. Metabolism and Pharmacokinetics Considerations

Pharmacokinetics considerations are important in determining common mechanisms of toxicity in a candidate set of chemicals. Information on the disposition of a chemical helps to elucidate issues of target site dose delivery. The study of the biotransformation of the chemicals will determine if a putative common toxic metabolite or its precursor are produced.

As will be discussed below, the candidate chloroacetanilides have many metabolic similarities, as well as some differences.

1. Absorption

Absorption of these chemicals after oral dosing is almost complete or at least very extensive. Data for **Propachlor** indicate that at least 68% of the dose is absorbed after oral dosing; oral absorption of the other compounds reaches values of 90% of the dose or more.

2. Tissue Distribution

Following oral dosing, radioactivity from the [C^{14}]-labeled parent chloroacetanilide and/or its metabolites is distributed extensively through all examined organs in rats.

The radioactivity is seen to bind extensively (up to 3% of the dose) to red blood cells (RBC) producing blood/plasma ratios of radioactivity of 18-315 for **Acetochlor**, **Butachlor**, and **Alachlor**. Likewise, **Metolachlor** produced ratios of RBC/liver protein greater than 11. The nature of the bound material is not known.

In the case of **Alachlor** levels of radioactivity in the non-glandular stomach exceeded those in the glandular stomach. As the dose decreased, the non-glandular stomach showed a decrease in percent of dose present, while the glandular stomach showed minor decreases in percent of dose of **Alachlor**-derived radioactivity.

Studies using whole body autoradiography (WBA) indicate that radioactivity from radiolabeled **Acetochlor**, **Alachlor** and **Metolachlor** is distributed to the **nasal turbinates** in the rat. Additional experiments with radio-labeled **Alachlor** indicate that distribution to the nasal turbinates is strain- and species- specific to the rat and not observed in mice, hamsters or squirrel-monkeys.

- Sprague-Dawley rats were administered **^{14}C-Acetochlor** in the diet at levels of 1750 or 5000 ppm. The animals were sacrificed after 14 days on the diet for whole body autoradiography (WBA) and microautoradiography. WBA revealed significant localization of radioactivity in the nasal turbinates. Micro radioautography in high-dose rats showed intense localization in the Bowman's glands, a lower degree in the olfactory surface and no evidence of localization in the respiratory epithelium. In low-dose animals only slight to moderate localization was seen in the Bowman's glands.

- Male Sprague-Dawley rats received 5 consecutive daily doses of the ^{14}C-secondary sulfide metabolite of **Acetochlor** by gavage. Rats were sacrificed at 1 or 5 days after the last dose for examination by WBA. Examination of radioautographs from animals sacrificed one day after dosing show high levels of radioactivity in the intestinal contents & liver, nasal turbinates, and lining of the tongue. At 5 days after dosing in addition to residual radioactivity in the stomach and intestinal contents, there was clear localization in the nasal turbinates, radioactivity in surrounding areas was greatly diminished. Micro radioautography showed that the label was concentrated in the Bowman glands of the nasal turbinates.

- Female Long-Evans rats, female CD-1 mice, and male squirrel monkeys were dosed with single oral doses of **^{14}C-Alachlor** at levels of 7, 70 or 700 mg/kg. Using WBA, radioactivity was found in liver, kidney, nasal vibrissae, body hair, oral strctures, and periorbital fat of all species. At 5 days, accumulation of labeled material was significant in the nasal turbinates of the rat, less in the mouse, and absent in the squirrel monkey.

- Similarly, female Sprague-Dawley, Long-Evans, and Fisher 344 rats and female syrian hamsters were given single oral doses of **^{14}C-Alachlor** at 7 or 70 mg/kg and tissue distribution was studied by WBA. At 24 hours post dosing all three strains of rat showed radiolabel in the highly perfused tissues. Nasal localization was observed in all three strains, but was most apparent in the Long-Evans strain. At no time was there any label in the nasal tissues of the Syrian Hamster.

- Single oral doses of 0.7 or 7.0 mg/kg **^{14}C-Alachlor methylsulfide** (a metabolite of **Alachlor**) given to female Long-Evans rats and the tissue distribution of radioactivity was studied by WBA. At 1-day post dose, radioactivity was observed in the intestines, stomach, and nasal turbinates. At 5 days localization of [^{14}C] was still evident in the nasal turbinates.

- Single oral doses of 7 or 70 mg/kg ^{14}C-diethylaniline (metabolite of **Alachlor**) were given to female Sprague-Dawley and CD-1 mice. At one day after dosing radioactivity was present in the major tissues of both rats and mice. Nasal localization was evident in the rat but not the mouse.

3. Excretion

Following oral dosing of rats with [C^{14}]-labeled chloroacetanilide the ratio of urine/feces excretion varies among the parent compounds, reflecting the differences in tissue distribution and biotransformation of the parent compound. Thus, the ratio of urinary excretion to feces excretion is close to 1 for **Alachlor** and **Metolachlor**, greater than 1 for **Acetochlor** and **Propachlor**, and less than 1 for **Butachlor**.

A large fraction of an oral dose of these five chloroacetanilides is conjugated with glutathione (GSH) and excreted to the intestine via the bile. While in the intestine the GSH conjugates excreted in the bile undergo further biotransformation followed by partial reabsorption through the intestinal wall, constituting a cyclic process of enterohepatic circulation. This process of enterohepatic circulation is significant in generating and facilitating the distribution of toxic metabolites.

4. Biotransformation

All five chloroacetanilides undergo extensive biotransformation in rats. Amounts of untransformed parent compound range from undetectable to 8% or less in feces. Numerous metabolites have been detected in numbers ranging from at least 11 in urine of **Propachlor**-treated rats up to 40 in urine of **Butachlor**-treated rats.

As expected from the SAR considerations, these chloroacetanilides undergo GSH conjugation at the chloroacetyl group, N-dealkylation, oxidative metabolism of the N-alkyl group, and in some cases oxidative metabolism or the ring alkyl groups.

5. Glutathione Conjugation and Benzoquinone imine Formation

Glutathione (GSH) conjugation at the chloroacetyl group is the major pathway of biotransformation for these compounds. GSH conjugation is of importance in interpreting the toxicity of these compounds. Products of further biotransformation of the GSH conjugates of **Acetochlor**, **Alachlor** and **Butachlor**, the respective electrophilic 2,6-dialkylbenzoquinone imines **(DABQI)**, have been associated with the production of nasal turbinate tumors in the rat.

The current working hypothesis for the induction of nasal tumors by **Alachlor** in rats proposes that **Alachlor** conjugates with GSH and is excreted in the bile. Subsequent biotransformation of the conjugate to a series of sulfur-containing products, followed by enterohepatic circulation of these products creates a pool of metabolites that are delivered to the nose where they undergo further biotransformation to tissue-reactive and toxic metabolites. Metabolism by nasal enzymes, results in formation of **DEBQI** (Figures 3 and 4), an electrophile, which binds to cellular proteins, producing cytotoxicity and regenerative cell proliferation. If cytotoxicity and cell proliferation is sustained, neoplasia eventually results.

As shown in Figure 3, a methyl sulfide metabolite of Alachlor is converted to 2,6-diethylaniline (2,6-DEA) following arylamidase action. 2,6-DAA is activated via a phenol metabolite precursor (4-amino-3,5-diethylphenol) to DEBQI (Figure 4). Although the 4-amino-3,5-diethylphenol has not been identified in excreta of rats dosed with **Alachlor**, this phenol has been formed <u>in vivo</u> in Long-Evans rats dosed with the methyl sulfide metabolite of **Alachlor** (Figure 3), appearing in urine as the sulfate (Figure 5) in quantities of 0.9-1.7% of the dose.

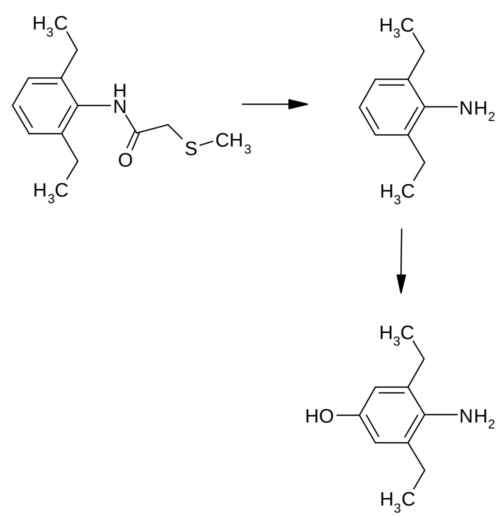


Figure 3. Formation of a
4-aminophenol metabolite from the methylsulfide metabolite of **Alachlor**.

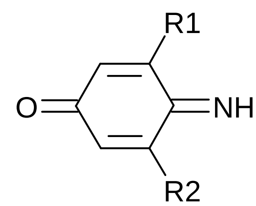


Figure 4. Structure of a dialkyl-benzoquinone imine. R1 and R2 are H or alkyl. For **Alachlor** R1 and R2 are -ethyl.

Independent work of Jeffries et al. [Chem. Res. Toxicol. *11*:353-359 , 1998] confirms these observations. These authors showed that the urine of S-D rats, 0-6 hours after intraperitoneal dosing with **Alachlor** yields a derivative that is diagnostic of the *in-vivo* formation of the diethyl-benzoquinone imine from **Alachlor.**

Data for the other members of the candidate group indicate that some of them, **Butachlor** and **Propachlor**, are metabolized to the corresponding 4-aminophenol derivative, a precursor of a benzoquinone imine.

In the case of **Butachlor**, although no 4-amino-3,5-diethylphenol or its sulfate were identified after oral dosing of rats, 4-amino-3,5-diethylphenol sulfate (Figure 5) has been identified in urine and feces of S-D rats dosed iv with a single dose of [^{14}C]Butachlor at 1, 10, or 100 mg/kg. Levels in urine were 1.9-2.5% of the dose in males and 1.1-2.0% of the dose in females. 4-Amino-3,5-diethylphenol can be activated to the benzoquinone imine shown in Figure 4. It is noted that its sulfate conjugation product, 4-amino-3,5-diethylphenol sulfate, was sought but not found in urine or feces of rhesus monkeys.

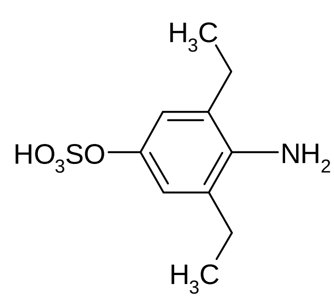


Figure 5. A **Butachlor** metabolite: 4-amino-3,5-diethylphenol sulphate

In the case of **Propachlor**, the N-acetyl derivative of the 4-aminophenol derivative and its glucuronide were found in urine of rats dosed orally with Propachlor. Data in the literature indicate that N-acetyl-4-aminophenol can be activated to a benzoquinone imine and has been found to cause necrosis in rat and human liver.

In the case of **Acetochlor**, the mercapturic acid conjugate of N-de-alkylated **Acetochlor** (Figure 6) is metabolized to a methyl sulfoxide. This observation allows the postulation of a putative Acetochlor methyl sulfide intermediate (not in the figure) that may also be eventually metabolized to a quinone imine. As in the case with Alachlor, experiments with **Acetochlor**-dosed rats, corroborate this contention indicating that this putative S-methyl intermediate metabolite can be metabolized and eventually converted to a 3-methyl-6-ethyl- benzoquinone imine. Structures of adducts to proteins isolated from nasal epithelium of rats dosed with ^{14}C-**Acetochlor** or ^{14}C- Acetochlor methyl sulfide indicate that this conversion to the benzoquinone imine takes place *in vivo* in the rat.

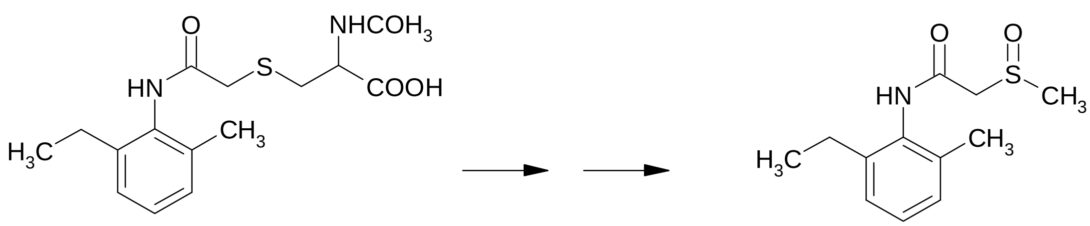


Figure 6. Sulfoxide formation from the mercapturic acid derivative of **Acetochlor**. A postulated secondary methyl sulfide intermediate metabolite of **Acetochlor** is not depicted (see Figure 3 for the analogous secondary methyl sulfide of **Alachlor**).

In the case of **Metolachlor**, 2,6-dialkylaniline (a possible benzoquinone imine precursor) has been detected in rats, however it has been at less than 0.00055% of the dose.

Independent work of Jeffries et al.(1998)confirms that indeed **Acetochlor**, **Butachlor**, and **Metolachlor** in addition to **Alachlor** may convert in vivo to their respective benzoquinone imines. These authors showed that the urine of S-D rats, 0-6 hours after intraperitoneal dosing with **Alachlo**r, **Acetochlor**, **Butachlor** or **Metolachlor** yields derivatives that are diagnostic of the *in-vivo* formation of dialkyl-benzoquinone imine from the respective parent chloroacetanilides.

6. Other Reactions

As discussed above, metabolic products include dealkylated materials resulting in secondary amines. The toxicological significance of these materials is not clear.

IV. Mechanisms of the Common Toxic Effects

This section depicts some of the postulated mechanisms for the common toxic endpoints by which the chloroacetanilides might be grouped. In particular, these sections review tumorigenesis in the nasal turbinates and the thyroid follicular cell as studied with **Acetochlor** and **Alachlor**. Mechanisms of stomach tumorigenesis resulting from treatment with **Butachlor** are also discussed. No mechanism is suggested as causing liver toxicity.

A. Mechanistic Aspects of Nasal Turbinate Tumorigenesis in Rats

This Section summarizes the postulated mechanism for the formation of nasal tumors as investigated initially for **Alachlor.**

The mechanism whereby chloroacetanilide pesticides produce nasal turbinate tumors in rats has been extensively investigated, initially for **Alachlor**. Subsequently, experiments with **Acetochlor** showed a similar mechanism, i.e., the formation of a benzoquinone imine intermediate that binds to nasal tissue protein. These studies are discussed below.

In Long-Evans rats of both sexes, **Alachlor** is metabolized to the glutathione conjugate which is excreted into the gut through the bile. In the gut, enteric bacteria metabolize the conjugate to the thiol conjugate, with subsequent S-methylation of the thiol. The product of this reaction, the methyl sulfide, is re-absorbed into the systemic circulation where conversion to the secondary sulfide occurs. Hydrolysis of the secondary sulfide by arylamidase produces the diethylaniline metabolite of **Alachlor.** Oxidation of the diethylaniline metabolite produces the putative toxic metabolite 2,6-diethyl-benzoquinone imine (DEBQI). DEBQI binds to cellular protein resulting in eventual cell death. Ensuing regenerative cell proliferation can then lead to neoplasia through fixation of spontaneous neoplasms.

Registrant's data for **Alachlor** show binding of the DEBQI metabolite to rat nasal protein at doses which deplete hepatic glutathione and which cause nasal tumors, supporting a non-genotoxic mode of action for nasal tumorigenicity. Data for **Acetochlor** also show binding of the corresponding dialkyl-benzoquinone imine at a dose that produces nasal tumors in S-D rats. Independent studies by Jefferies et al.(1998) have presented direct evidence for the in vivo production of benzoquinone imine metabolites from **Acetochlor**, **Alachlor**, **Butachlor** and **Metolachlor** in male S-D rats.

Although experimental evidence for binding of an alkyl-benzoquinone imine to nasal proteins is not available for **Metolachlor** or **Butachlor**, evidence for the metabolic formation of a benzoquinone imine metabolite from these compounds exists and was discussed in section III.C.5. In the case of propachlor, although it does not form nasal turbinate tumors, the 4-aminophenol precursor of the benzoquinonone imine has been identified as a metabolite.

B. Mechanistic Aspects of Stomach Tumors in Rats

This Section summarizes the postulated mechanism for the formation of stomach tumors as investigated for **Butachlor**.

Mechanistic aspects of stomach tumor formation as a result of chloroacetanilide administration to rats have been derived largely from studies conducted on **Butachlor** and **Alachlor**.

Data reviewed for **Butachlor** show that at a high dose of 213 mg/kg/day given to female S-D rats for 22 months, cell proliferation in the neck and base regions of the fundus glands of the stomach was significantly increased, while mucosal thickness was decreased in relation to untreated rats. Gastric pH and serum gastrin levels were also increased at the high dose. Stomach tumors were observed, with small (early) tumors composed of enterochromaffin like endocrine cells of glandular tissue. Larger tumors (late neoplasms) were of mixed cell types, with some predominantly endocrine-like and others containing endocrine like cells which were poorly differentiated. Advanced tumors may have progressed from well differentiated neuroendocrine lesions to more undifferentiated neoplasms, or there may have been more than one cell type of origin for a tumor.

Based on the above data, the mechanism proposed for stomach tumor formation involves atrophy of the fundic mucosa following high dose exposure and consequent loss of the deeper elements of the mucosal epithelium. Mucosal atrophy leads to compensatory cell proliferation in the fundic mucosa, while loss of parietal cells results in extensive gastric hypochlorhydria and a subsequent increase in gastric pH. The increase in gastric pH induces excessive production of gastrin, resulting in elevated serum gastrin. The trophic effect of gastrin on the enterochromaffin-like and fundic stem cells further drives a sustained cell proliferation which ultimately results in induction of gastric neoplasms.

C. Mechanistic Aspects of Thyroid Follicular Cell Tumors

This Section summarizes the postulated mechanism for the formation of thyroid follicular tumors as investigated initially for **Alachlor**. Subsequent work has produced experimental evidence supporting the same mode of action for **Acetochlor** and **Butachlor**.

The mechanistic information in support of thyroid tumor induction was developed initially from data for **Alachlor**. The mechanism whereby thyroid tumors arise in Long-Evans rats from the chronic administration of **Alachlor** is based on the induction of hepatic uridine 5'-diphospho glucuronyltransferase with a subsequent decrease in circulating T3 and T4 levels, a subsequent increase in circulating TSH, and eventual hyperplasia and neoplasia of the thyroid based on exposure of the rat thyroid to elevated and sustained levels of TSH. A mechanistic study conducted by the registrant for **Alachlor** included 5 groups of rats which were administered **Alachlor** in the diet at 126 mg/kg/day for up to 120 days. An additional group of 20 rats was exposed to **Alachlor** for 60 days and then untreated diet for another 60 days to determine reversibility. The results of this study showed consistent increases in liver weight, thyroid weight, and activity of UDPGT as well as elevations in serum TSH. Changes in T3 and T4 were inconsistent. Elevations in TSH, liver weight, and thyroid weight were reversible upon cessation of exposure. The data from this study show that **Alachlor** appears to act in a manner similar to that observed with a wide range of chemicals which are inducers of hepatic microsomal enzymes, but which are not mutagenic and produce neoplasia only at high doses.

V. Weight of the Evidence Evaluation for Grouping Chloroacetanilides by a Common Mechanism of Toxicity

Table 2 lists the various parameters that are considered to be relevant in defining those chloroacetanilides that can be considered to have a common mechanism of toxicity. The relevant lines of evidence for grouping are discussed in the following pages.

Table 2. Evidence used in grouping/excluding chloroacetanilide pesticides by common mechanism of toxicity.[a]

Parameter	Acetochlor	Alachlor	Butachlor	Metolachlor	Propachlor
Nasal tumors in rats	Yes	Yes	Yes	Tumors reported but p>0.05. Chemical distributes to nasal turbinates in L-E rats	No
Forms quinone imine (QI) metabolite	Yes	Yes	Yes	Possible (but levels of 2,6-dialkylaniline are very low)	Probably. Forms the a p-aminophenol derivative, which can be activated to a QI
Nasal turbinate cell Proliferation	Yes	Yes	Yes	No Data	No Data
Nasal tumors based on QI	Yes	Yes	Yes	Nasal tumors, but not statistically significant	No
Thyroid Follicular Cell tumors in rats	Yes	Yes	Yes	No	No (C-cell tumors only)
Thyroid tumors based on induction of microsomal enzymes	Yes (T4 -UDPGT induced)	Yes (T4 -UDPGT induced)	Yes (T4 -UDPGT induced)	No	Not known. But compound decreases sleeping time in rats
T4 / T3 and TSH distubances	Yes	Yes	Yes	No Data	No Data
Liver tumors	Statistically significant increase in the incidence of combined hepatocytic adenomas/carcinomas was observed in male CD-1 mice only at the high dose	No	No	Liver adenoma/carcino. in rats: trend and pairwise in females, trend in males	Significant increase in hepatocellular tumors at high dose in male mice

A. Rat Nasal Turbinate Tumors

1. Tumor incidence

Three members of the candidate series **Acetochlor, Alachlor, and Butachlor** show tumors of the nasal turbinates in both sexes of rats. Although **Metolachlor** produced nasal turbinate tumors (1 adenocarcinoma, 1 fibrosarcoma at the high dose), the incidence was not statistically significant and the chronic bioassay was considered to have been done at too low of a dose. For **Propachlor**, chronic studies do not indicate formation of nasal tumors by this compound.

2. Benzoquinone imine Formation and Binding to Tissue Proteins

Whole body autoradiography studies performed in rats with radiolabeled **Acetochlor** indicates that this compound and/or its metabolites distributes to the nasal turbinates; microradioautography revealed that this distribution is largely to the Bowman's glands and to the olfactory epithelium, with no distribution to the respiratory epithelium. Additional studies performed with the ^{14}C- Secondary Sulfide metabolite of **Acetochlor**, indicate that the compound or its metabolites also distributes to the nasal turbinates (largely to the Bowman's gland) Analysis of protein binding in the nasal turbinates showed the presence of an ethyl-methyl-benzoquinone imine-derived protein adduct after treatment with the ^{14}C- Secondary Sulfide metabolite of **Acetochlor**. This observation shows that a metabolite of **Acetochlor** can be metabolized to a dialkyl-benzoquinone imine and bind to tissue proteins. Dialkyl-benzoquinone imines are regarded as toxic metabolites, and they are considered to bind to cellular proteins, resulting in cell death. The ensuing regenerative proliferation of the nasal epithelium is regarded as responsible for fixation of spontaneous mutations and the eventual formation of nasal tumors

Mechanistic studies of nasal tumorigenesis performed with **Alachlor** and its metabolites indicate that a sulfur metabolite of **Alachlor** is also distributed to the nasal turbinates of the rat followed by its subsequent metabolic conversion in situ to a reactive dialkyl-benzoquinone imine, 2,6-diethylbenzoquinone imine.

Butachlor is likely to form a benzoquinone imine, based on the identification of its precursor among the metabolites of the rat. **Metolachlor** appears to have the potential to form a benzoquinone imine, based on the identification of small amounts of the precursor 2,6-dimethyl aniline. **Propachlor** does form the precursor to a benzoquinone imine; however, there is no evidence that it is distributed to the nasal turbinates in the rat.

3. Nasal Cell Proliferation

An essential step in the mechanism of toxicity postulated for formation of nasal turbinate tumors in rats is the regenerative cell proliferation of the nasal epithelium that can then lead to neoplasia if sustained.

- ❑ Groups of male Sprague- Dawley rats (9-10/dose) were administered **Acetochlor,** at levels of 0, 10.4, 91.9, or 270.3 mg/kg bw/day for up to 160 days. Increases in cell proliferation in the nasal epithelial cells in the olfactory region were statistically significant over control values as early as 60 days of treatment and persisted for up to at least 160 days. The effect was dose-related, reaching statistical significance at 91.9 mg/kg bw/day in the olfactory epithelium. Statistically significant increases in incidences of nasal turbinate tumors were seen in a 2-year chronic study in female S-D rats at 92.1 mg/kg/day and in males at 66.9 mg/kg/day. Thus, there is a dose-response concordance for cell proliferation and induction of nasal turbinate tumors.

- ❑ Groups of 20 female Long-Evans rats were administered **Alachlor**, at levels of 0, 1, 126, or 252 mg/kg bw/day for up to 60 days. Cell proliferation in the nasal epithelial cells in the respiratory olfactory junction was increased after 10 days of dosing and showed a dose-related trend. After 30 days of dosing, an increase in cell proliferation was observed which was dose-related and statistically significant at the highest dose. At 60 days, the increase was statistically significant at 126 and 252 mg/kg/day. Statistically significant increases in incidences of adenomas and combined adenomas/carcinomas in nasal turbinates were seen in a 2-year chronic study in Long-Evans rats at 42 mg/kg/day. In another chronic study conducted at dose levels of 0.5-15 mg/kg/day, only adenomas were statistically significantly elevated at the high dose. No cell proliferation studies have been conducted at these low doses.

- ❑ In another series of experiments, groups of female Long-Evans rats were administered **Alachlor** in the diet at dose levels of 0, 0.5, 2.5, 15, 42, or 126 mg/kg/day for 60 days. Cell proliferation was statistically significantly increased in the olfactory region of the nasal turbinates at doses of 42 and 126 mg/kg/day. No increases were seen in the respiratory region. The effect was reversed after 60 days withdrawal from **Alachlor**. It is noteworthy that no proliferative effect was seen in CD-1 mice dosed with alachlor in the feed for 10 or 60 days at levels up to 260 mg/kg/day. Alachlor does not produce nasal tumors in mice.

- ❑ In experiments with **Butachlor**, groups of female Sprague Dawley rats were treated at levels of 0, 6.6, 66.1, or 212.9 mg/kg/day for up to 20 months. Increases in cell proliferation in the nasal epithelial cells in the olfactory and respiratory regions were statistically significant over control values as early as 60 days of treatment and persisted for up to at least 20 months. The effect was dose-related, reaching statistical significance at 66.12 mg/kg/day in the olfactory epithelium. Statistically significant increase in incidences of nasal turbinate tumors were seen in a 2-year chronic study in female S-D rats at 58.5 mg/kg/day and in males at 45.6 mg/kg/day. Therefore, there is dose-response concordance for cell proliferation and induction of nasal turbinate tumors.

B. Rat Thyroid Follicular Cell Tumors

1. Tumor Incidence

Thyroid follicular cell tumors have been reported for **Acetochlor, Alachlor, and Butachlor** in rats. In S-D rats tested with **Propachlor** at doses of up to 500 ppm in the diet for 104 weeks, the incidence of thyroid neoplasia was limited to C-cell tumors, not to follicular cell tumors. Thyroid tumors have not been reported for other compounds of the candidate group.

2. Microsomal Enzyme Induction

Mechanistic studies of thyroid follicular cell tumorigenesis performed with **Alachlor** indicate a hormonally-mediated mechanism for thyroid neoplasia. Administration of Alachlor results in induction of microsomal hepatic UDPGT activity, which produces increased clearance of thyroid hormone, T_4. Decreased levels of T_4 would result in increased levels of thyroid stimulating hormone (TSH). Increased levels of TSH would result in the hyperplastic and eventually tumorigenic response of the thyroid.

Mechanistic data for **Acetochlor** and **Butachlor**, likewise indicate a hormonally-mediated mechanism for the thyroid neoplasia induced by these compounds. Administration of **Acetochlor** and of **Butachlor** in the diet results in statistically significant increases in relative liver weights, relative thyroid weights, and in increases in microsomal hepatic UDPGT, T_4 and TSH. For **Propachlor**, thyroid C-cell tumors have been observed after chronic high dose administration of the compound. However, as C-cells are engaged in calcitonin production and not T3/T4 production, the mechanism of induction for these tumors is expected to be different. Additionally, **Propachlor** has been found to produce a decreased barbiturate-induced sleeping time in rats (a common indirect test of microsomal-enzyme induction).

C. Stomach Tumors in Rats

Butachlor and Alachlor showed tumors in the fundic region of the stomach. Additionally, basal cell tumors have been seen for **Acetochlor**, but statistical significance was not reached. Although 1 gastric carcinoma was observed for **Propachlor** at the highest dose, the tumor was present in the pyloric region of the rat stomach.

Although **Butachlor** and **Alachlor** and maybe **Acetochlor** (at high doses) could be clustered based on the induction of stomach tumors, some limitations exist. Primarily, the diagnosis of the specific tumor types resulting from administration of the various chloroacetanilide herbicides has not been consistent. This is based on the lack of detailed histopathologic analysis of stomach tissue from the various studies conducted. In the case of **Acetochlor**, "basal cell tumors" were described, while in the case of **Alachlor**, "mixed cell tumors" were described. Although initiation / promotion studies with both **Butachlor** and **Alachlor** indicate that both chemicals act as promoters of tumorigenesis through a hormonally mediated, non-genotoxic mechanism, the evidence for support of a common mechanism is not definitive, especially in light of the FIFRA Scientific Advisory Panel's determination that for **Alachlor**, "the evidence presented that the carcinomas resulting from Alachlor were...carcinoids, not adenocarcinomas or gastric sarcomas, which are unrelated to the proposed gastrin-induced effect." EPA agrees with the SAP's conclusions.

Thus at this time, the formation of gastric tumors, **is not** a suitable toxic endpoint for grouping the chloroacetanilides.

D. Liver Tumors in Rodents

Examination of liver histopathology indicates that **Acetochlor**, **Metolachlor** and **Propachlor** are associated with liver tumors in rodents in chronic studies. **Acetochlor** produced a statistically significant pairwise increase in combined hepatocellular adenomas/carcinomas in high-dose CD-1 male mice. **Metolachlor** produced statistically significant pair-wise increases in liver adenomas and combined adenomas/carcinomas in high-dose female rats and a statistically significant trend for liver tumors in male rats. **Propachlor** produced a statistically significant increase in hepatocellular tumors [adenomas, carcinomas, hepatoblastomas] in high dose male CD-1 mice.

VI. Conclusions on Common Mechanism

Examination of the above evidence indicates that the Candidate Series of chloroacetanilides can be clustered according to **three** grouping scenarios of varying degrees of validity based on decreasing weight of the evidence.

A. Nasal Turbinate Tumors

Acetochlor, Alachlor and Butachlor may be grouped together based on a common end-point, a known mechanism of toxicity for this endpoint. This grouping appears to have the strongest support for the three groupings discussed in this section.

Although **Metolachlor** does distribute to the nasal turbinates, and might produce a quinoneimine, it is not apparent that it shares the same target organs of toxicity as **Acetochlor, Alachlor and Butachlor.** At this time, therefore, there is insufficient information for including metolachlor in the common mechanism group. Although **Propachlor** does produce a precursor of a quinoneimine, there are no data to support its tumorigenicity to the nasal turbinates.

The grouping of **Acetochlor**, **Alachlor**, and **Butachlor** was presented to the FIFRA Scientific Advisory Panel (SAP)as a draft on March 19, 1997. The SAP agreed with the Agency's conclusion that there is sufficient evidence to support the proposed grouping for the nasal turbinate tumors.

B. Thyroid Follicular Cell Tumors

Acetochlor, Alachlor and Butachlor may be grouped together based on a common end-point and a known mechanism of toxicity (UDPGT induction). Data for all three chloroacetanilides exist (positive UDPGT induction, increased TSH, alterations in t3/t4 production, increased thyroid weights) to confirm that the postulated mechanism of action is indeed responsible for the effect.

In the case of **Propachlor**, the compound produces C-cell tumors of the thyroid. However, as C-cells are engaged in calcitonin production and not T3/T4 production, the mechanism of induction for these tumors is expected to be different than the one postulated for **Acetochlor**, **Alachlor**, and **Butachlor**. **Propachlor** has been found to produce a decreased barbiturate-induced sleeping time in rats, increased liver weights and hepatocellular hypertrophy (all consistent with microsomal enzyme induction). Thus, although **Propachlor** could qualify as a suspect member of this class, based on the possible induction of microsomal enzymes, there is no evidence of production of thyroid follicular cell tumors.

This grouping of chloroacetanilide pesticides was presented to the FIFRA SAP as a draft on March 19, 1997. The SAP recommended that regarding the thyroid tumors, even though the case study illustrated that a common mechanism could be used to group chloroacetanilides for the development of thyroid tumors, this endpoint should not be used in combining margins of exposure because the toxic effects were seen at doses that were considered to be excessive.

C. Formation of Liver Tumors

A third grouping could be attempted to include **Acetochlor, Metolachlor, and Propachlor**, based on the production of liver neoplasia in rodents. These chemicals can only be linked by structural similarity and a common toxic endpoint, liver tumors. There are no data demonstrating a common mechanism of action or of a common toxic species responsible for the effect. Thus there is an insufficient basis at this time for grouping these chemical by common mechanism of toxicity based on liver tumors.

VII. Recommendation for Grouping of Chloroacetanilide Pesticides Based on Common Mechanism of Action

The weight-of-evidence supports that only **Acetochlor**, **Alachlor**, and **Butachlor** be grouped by a common mechanism of toxicity for nasal turbinate tumors for purposes of a cumulative risk assessment. Although the available evidence contains evidence suggesting **Acetochlor**, **Alachlor**, and **Butachlor** may have a common mechanism of toxicity based on thyroid follicular cell tumors, this endpoint may not be suitable for risk assessment, because the toxic effects were noted at doses above the Maximum Tolerated Dose (MTD). Thus the weight-of-evidence supports that Acetochlor, Alachlor and Butachlor not be grouped based on thyroid follicular cell tumors for purposes of risk assessment. At this time there is inadequate data for grouping chloroacetanilides based on common mechanism of action for induction of stomach or liver tumors.

April 28, 1997

MEMORANDUM

SUBJECT: Transmittal of the Final Report of the FIFRA Scientific Advisory Panel Meeting for March 19 and 20, 1997.

FROM: Larry C. Dorsey
Designated Federal Official
FIFRA Scientific Advisory Panel

TO: Daniel M. Barolo, Director
Office of Pesticide Programs

Please find attached the final report of the FIFRA Scientific Advisory Panel(SAP) open meeting held in Arlington, Virginia on March 19 - 20, 1997. This report includes SAP findings on scientific issues discussed at the meeting concerning Toxicology Endpoint Selection Process, Inhalation Risk Assessments and the Combining of Margin of Exposures, Aggregate Exposure Methodology Issues, Common Mechanism of Action, Visual System Toxicity Testing of Organophosphates, and Standard Operating Procedures for Peer Reviews.

Attachment

cc: Lynn Goldman
Penny Fenner-Crisp
Stephen Johnson
Joseph Cara
Freedom of Information Office
Al Heier
Don Barnes
Vicki Ellis
Margaret Stasikowski
Stephanie Irene
Lois Rossi

Angela Auletta
Vanessa Vu

FEDERAL INSECTICIDE, FUNGICIDE, AND RODENTICIDE ACT
SCIENTIFIC ADVISORY PANEL
MEETING

A Set of Scientific Issues Being Considered by the Agency to Discuss and Evaluate the Common Mechanism of Toxicity.

The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel (SAP) has completed its review of a Common Mechanism of Toxicity for use in Combined Risk Assessment. The review was conducted in an open meeting held in Arlington, Virginia, on March 20, 1997. The meeting was chaired by Dr. Ernest E. McConnell. Other panel members present were: Dr. Leo Abood (University of Rochester); Dr. Charles C. Capen (Ohio State University); Dr. Michael L. Dourson (Toxicology Excellence for Risk Assessment); Dr. Richard Fenske (University of Washington); Dr. Charles H. Hobbs (Inhalation Toxicology Research Institute); Dr. Ronald J. Kendall (Clemson University); Dr. Harihara M. Mehendale (Northeast Louisiana University); Dr. Michele A. Medinsky (Chemical Industry Institute of Toxicology); Dr. Robert L. Peiffer, Jr., (University of North Carolina); Dr. James Render (Michigan State University); Dr. James A. Swenberg (University of North Carolina); Dr. Mary Anna Thrall (Colorado State University).

Public Notice of the meeting was published in the Federal Register on January 29, 1997.

Oral statements were received from:
Dr. Michael J. L. Clapp, ZENECA
Dr. Donald R. Saunders, American Crop Protection Association
Dr. John A. Todhunter, SRS International Corporation
Dr. David Wallinga, Natural Resources Defense Council
Dr. Alan G. E. Wilson, Monsanto

Written comments were received from:

American Crop Protection Association
Monsanto

GENERAL RESPONSE OF PANEL MEMBERS

QUESTIONS AND PANEL RESPONSES FOR COMMON MECHANISM OF TOXICITY SESSION

For the purposes of FQPA, mechanism of toxicity has been defined as the major steps leading to an adverse health effect following interaction of a pesticide with biological targets. All steps leading to an effect do not need to be specifically understood. Rather, it is the identification of the crucial events following chemical interaction that are required in being able to describe a mechanism of toxicity. Common mechanism of toxicity has been defined as cases where two or more chemicals produce or may be expected to produce adverse effects by the same crucial step(s). The Agency recognizes that fully developed molecular understanding of mechanisms by which pesticide chemicals exert adverse effects will not be available **in most cases.**

QUESTION 1:

For the purposes of FQPA, are the Agency's proposed definitions of mechanism of toxicity and common mechanism of toxicity consistent with current toxicological concepts?

SAP RECOMMENDATION

The Scientific Advisory Panel finds that the proposed definitions are useful and agrees with the overall approach for identifying common mechanisms of action; however, the Panel is of the opinion that the document could be improved in several ways. For example, the document should include a discussion or summary of the definition for mechanism of toxicity taken from the current published literature such as the most recent edition of Cassarett and Doull's Toxicology (page 35). As a general comment, the Panel recommends incorporation of references into the document where appropriate. The section of the document describing common mechanisms should also be expanded to include a discussion of the different levels of evidence for common mechanisms, and definitions of terms such as toxic endpoint, biological plausibility (least amount of data), mode of action (moderate amount of data), and ultimate mechanism of toxicity (most amount of data). Clarification of what the agency considers the biological target would also be useful. For example, is the target considered to be a specific molecule, cell or tissue, or does target refer to the whole organism? Biological interaction with a specific receptor or enzyme should be used as the starting point for grouping chemicals into a common mechanism.

Some concerns were raised regarding the use of the phrase "may be expected to produce." It would be most useful if the Agency could expand on the concept and include examples how this phase could be applied. The Agency should leave some flexibility in the guidelines to allow for situations where the toxic endpoint effect has not been demonstrated, but the established causative toxic metabolite intermediate has been. In such a case, there would be sufficient rationale to anticipate expected toxic effect and therefore not exclude such a chemical from the common mechanism grouping.

<u>QUESTION 2:</u>

The Agency plans to use a number of different elements of evidence in making decisions about mechanism of toxicity and whether a common mechanism applies to members of a group of pesticide chemicals. Usually included will be considerations of chemical structure, metabolism, types of toxicological effects and other data as appropriate.

> Determinations of whether a common mechanism of toxicity is operating will be based on all available information evaluated by a weight of evidence approach as demonstrated by the case study. How might this process be improved to make it more scientifically sound?

<u>SAP RECOMMENDATION</u>

The Panel strongly endorses the weight of evidence approach for determining presence or absence of common mechanism of action. The Panel encourages the Agency to incorporate peer reviewed scientific publications into the weight of evidence. Additional scientific evidence can be incorporated provided it is reliable and reproducible. Anecdotal information should not be used.

QUESTION 3:

Two basic strategies will be employed to determine whether a common mechanism of toxicity is operating.

a. **Structure based**: Begin with a group of pesticide chemicals that have one or more structural similarities. Proceed with an investigation of metabolism of the compounds. Determine whether there is a common biological effect. Ascertain the mechanism by which the effect is produced if information is available to do so. Judge whether the mechanism is common to chemicals in the group. Combine risks for those with a common mechanism.

b. **Mechanism based:** Determine the mechanism of individual pesticide chemicals that are or are not structurally related. Combine risks for those that have a common mechanism.

Comment on the merits of these two strategies to identify pesticide chemicals for inclusion in combined risk assessment.

SAP RECOMMENDATION

Chemicals with similar structures may not have the same mechanism of action. However, grouping chemicals with structural similarities can serve as a starting point or a convenient way to triage chemicals for examining the basis for common mechanisms. The important point is that whether the Agency begins by examining a group of chemicals with similar structures or a group of chemicals with a common mechanism, the weight of evidence approach must be applied for grouping chemicals with regard to a common mechanism of toxicity. Starting with a group of chemicals with similarities in structure and toxic endpoint would appear to be superior as an initial strategy to starting with a given mechanism. The Panel encourages the Agency to develop and use Structure Activity Relationship (SAR) information technology to enhance their effort to develop the common mechanisms approach.

Although, for a class of structurally homologous agents, it is appropriate to relate a toxic end point to a common mechanism of action, there are instances where some or all members of the class may have alternate mechanisms resulting in the same or different toxic end points. For example, in addition to their ability to form DNA adducts after bioactivation and formation of reactive electrophiles, xenobiotic carcinogens can also cause neoplastic growth promotion, cytotoxicity, inhibition of tissue growth regulation, peroxisome proliferation, endocrine modification, immunosuppression and/or sustained tissue ischemia.

The Scientific Advisory Panel finds that the use of SAR and QSAR (quantitative SAR) methodology is an important component for predicting common mechanisms in the absence of mechanistic data and for determining potential mechanisms by analyzing diverse model parameters. In recent years, a number of computerized structure-activity programs have been developed to model and analyze multiple or overlapping mechanisms with a single toxic end point. Such methodology can provide confidence levels on predictions in the absence of mechanistic data, as well as determine the potential mechanistic significance of diverse model parameters. A combination of comparative molecular field analysis (COMFA) and QSAR has been used to relate ecotoxicological data with the steric and electrostatic fields of chlorophenola for 16 different biological systems. The technique also allows prediction of values in the absence of missing ecotoxicological data. The Panel recommends that the Agency take necessary action to develop and acquire such technology to facilitate evaluation of pesticides through structure activity relationships as an initial approach.

QUESTION 4:

The case study for chloroacetanilides groups pesticide chemicals according to three grouping scenarios based upon varying degrees and quality of evidence.

a) For the nasal tumors, a well-developed understanding of the underlying mechanism is available for one member of the class and appears to be applicable to others. For these pesticide chemicals, precursors to the putative, critical metabolite quinone imine have been identified for each chemical.

b) For the thyroid tumors, a hypothetical mechanism has been developed for one chemical, linking the response to concurrent changes in microsomal enzymes that metabolize thyroid hormone. Effects on the liver for other members of the group are consistent with an influence on microsomal enzymes, suggesting a common mechanism of toxicity.

c) For pesticide chemicals inducing liver tumors, there is no specific knowledge of a mechanism of action. However, the pesticide chemicals are linked by structural similarity and common toxic endpoints.

The Agency believes that there is sufficient evidence to support groupings a and b, but insufficient evidence for paragraph c above. Are these groupings consistent with the Agency's proposed methodologies and definitions?

SAP RECOMMENDATION

The case study provided by the Agency on an approach for determining common mechanism of action was excellent, well-presented, and very appropriate. The Panel suggests that the Agency develop an equally illustrative example for determining a common mechanism of action for a group of chemicals with a non-cancer endpoint.

The Scientific Advisory Panel agrees with the Agency's conclusion that there is sufficient evidence to support the proposed groupings for the nasal tumors. Regarding the thyroid tumors, even though the case study illustrated a common mechanism could be used to group certain chemicals for the development of thyroid tumors, the Panel recommends that this endpoint not be used in combining margins of exposure because the toxic effects were noted at doses above the Maximum Tolerated Dose (MTD). While the full range of doses employed can be used to determine common mechanisms, endpoints occurring solely at doses above the MTD should not be used in risk assessments.

QUESTION 5:

The Agency recognizes that scientific judgment is critical to the determination of whether a mechanism of toxicity has been identified and whether a mechanism is common across chemicals. To ensure that Agency decisions are based on good scientific principles, at least initially, individual assessments on chemical classes will be subjected to external peer review. Comment on the adequacy of this plan in ensuring high scientific standards.

SAP RECOMMENDATION

The Panel strongly endorses the use of peer review to support or reject the Agency's position regarding a common mechanism of action for selected groupings of chemicals. Peer review will be especially important initially when methodologies to combine chemicals into common groupings are being developed. The panel recognizes that while some groupings such as that provided in the case study for nasal tumors will be relatively straightforward, many more groupings will be in “grey zones” that are less well defined. It will also be important for the Agency to interact with a wide range of other groups to develop paradigms to move forward with the grouping of chemicals with the same mechanism of toxicity. The Panel supports the draft guidelines that the Agency has outlined so far for determining common mechanism of toxicity.

PEER REVIEW SELF-ASSESSMENT AND VERIFICATION SURVEY

Office Identification

AA-ship/Region: Office of Prevention, Pesticides, and Toxic Substances

Office/Division: Office of Pesticide Programs / Health Effects Division

Self-assessment conducted by: Alberto Protzel, Ph.D.

Date self-assessment completed: August 8, 1999

Division Director (or other level) signature: Margaret Stasikowski, Director

Product Identification

Product name: **Guidance for Establishing a Common Mechanism of Toxicity for Use in Combined Risk Assessments. Case Study: The Grouping of a Series of Chloroacetanilide Pesticides Based on A Common Mechanism of Toxicity**

Peer Review Organizer: Larry Dorsey

Review Mechanism: Scientific Advisory Panel (SAP)

Date Peer Review Completed: March 20, 1997

Product description: On March 20 1997, the FIFRA Scientific Advisory Panel (SAP) was asked to comment on a proposed guidance for establishing a common mechanism of toxicity for use in combined risk assessments [Attachment 1] and to comment on the application of this guidance to the grouping of a series of chloroacetanilide pesticides based on a common mechanism of toxicity [Attachment 2]. In connection with the proposed protocol, the SAP was asked to address five specific questions [Attachment 3].

Outcome (Please circle or bold appropriate descriptor and provide any relevant comment(s).)

1. Overall peer reviewer assessment of product:
   a. **Highly satisfactory** b. Generally satisfactory c. Mildly critical d. Highly critical

   The SAP Panel found that the proposed definitions were useful and agreed with the overall approach for identifying common mechanisms of action. The Panel strongly endorsed the use of the weight of evidence approach. The Panel supported the draft guidelines that the Agency had outlined so far for determining a common mechanism of toxicity. The Panel considered that the case study provided by the Agency was excellent, well presented and very appropriate.

2. Overall EPA office/region assessment of peer review comments:
a. Highly useful b. Generally useful c. Limited use d. Harmful

3. Overall office/region self-assessment of compliance with peer review SOPs:
a. Full compliance b. Partial compliance c. Little or no compliance

4. Explain significant departures, if any from last year's Appendix D plan or SOPs.
None.

Process

1. Attach list of reviewers with their affiliations. How were the peer reviewers identified? How were expertise, affiliation, conflict of interest, etc., considerations made?

A list of the reviewers convened for this SAP meeting is attached (Attachment 3, page 35). This list includes the chairman of the SAP plus the *ad hoc* panel of scientific experts. The experts were selected based on their areas of expertise and availability to participate in this review.

In reference to conflict of interest and other considerations, each *ad hoc* expert who agreed to serve received the following documents: EPA Ethics Advisories 88-6 and 85-16, SF-278 Executive Personnel Financial Disclosure Report, 40 CFR Part 3 - Employees Responsibilities and Conduct and U.S. EPA Guidance on Ethics and Conflicts of Interest (2/84).

This documentation explains (1) that the individual is appointed as a Special Government Employee in order to serve on the FIFRA SAP, and (2) the laws and regulations which are applicable to him/her in that capacity. Once an individual has agreed to serve on the FIFRA SAP as a panel member or an *ad hoc* expert, he/she must submit all applicable employment and tax forms, as well as a Financial Disclosure Report and EPA Form 3120-1 "Confidential Statement of Employment and Financial Interests" to the Executive Secretary. All panel members and *ad hoc* experts are briefed prior to their first SAP meeting on the conflicts of interests laws and regulations by the Designated Agency Official or alternate. A record of all such briefings is kept by the Executive Secretary.

2. Attach a copy of the charge. How was the charge to the reviewers prepared?

A copy of the charge to the SAP is attached (Attachment 3). It consisted of five specific questions listed in Attachment 3 and two briefing papers consisting of the Guidance for Establishing a Common Mechanism of Toxicity (Attachment 1) and the Case Study for Grouping a Series of Chloroacetanilides based on Common Mechanism of Toxicity (Attachment 2).
Elizabeth Doyle, Ph.D., had the lead in preparing the Guidance for Establishing a Common Mechanism of Toxicity (Attachment 1) and Alberto Protzel, Ph.D. had the lead in preparing the Case Study for Grouping a Series of Chloroacetanilides based on Common Mechanism of Toxicity (Attachment 2).

3. How was the decision made to review this product?

HED chose to present these issues to the SAP and the public for review because of the importance of this issue in performing cumulative risk assessments of pesticides and other substances that have a common mechanism of toxicity, as required by Food Quality Protection Act (FQPA) of August 3, 1996.

4. How was the review mechanism chosen?

The SAP was created by the Federal Insecticide, Fungicide, and Rodenticide Act in the 1970s to judge the impact of proposed OPP regulatory decisions on health and the environment. In practice, however, the role of the SAP has been expanded to a peer review body for current scientific issues which may influence the direction of OPP's regulatory decisions. It is composed of seven members who are selected on the basis of their professional qualifications to assess the impact of pesticides on health and the environment. Additional scientists are available as *ad hoc* members of the SAP. These scientists are chosen because of their unique expertise not covered by the regular panel members.

5. Identify location of the peer review file and person(s) responsible. Are materials available upon request? Is the file kept in a centralized location or maintained separately with a centralized "pointer file" identifying its location?

Larry Dorsey, Designated Federal Official for the SAP, maintains the SAP files in a centralized location. The peer review self-certification package file is maintained by the OPP Peer Review Coordinator, Henry Spencer.

6. How did the product <u>change</u> to accommodate the review comments?

Several modifications (Steve DeVito, Ph.D. had the lead) , discused below, were made to the draft Guidance document to accomodate the review comments. The modifications suggested by the SAP, in addition to modifications resulting from the response to public comments, are embodied in a public document *Guidance for Identifying Pesticide Chemicals and other Substances that have a Common Mechanism of Toxicity*, dated January 29, 1999. This document is available from the Agency as Fax-On-Demand Item No. 6055. Fax Number: (202)401-0527.

Comments by the SAP were addressed as follows:

<u>QUESTION 1:</u> For the purposes of FQPA, are the Agency's proposed definitions of mechanism of toxicity and common mechanism of toxicity consistent with current toxicological concepts?

The SAP recommended that: " ....the document should include a discussion or summary of the definition for mechanism of toxicity taken from the current published literature such as the most recent edition of Cassarett and Doull's Toxicology (page 35). As a general comment, the Panel recommends incorporation of references into the document where appropriate. The section of the document describing common mechanisms should also be expanded to include a discussion of the different levels of evidence for common mechanisms, and definitions of terms such as toxic endpoint, biological plausibility (least amount of data), mode of action (moderate amount of data), and ultimate mechanism of toxicity (most amount of data). Clarification of what the agency considers the biological target would also be useful. For example, is the target considered to be a specific molecule, cell or tissue, or does target refer to the whole organism? Biological interaction with a specific receptor or enzyme should be used as the starting point for grouping chemicals into a common mechanism ”

The Agency responded by including definitions of the terms requested by the SAP or of related terms in pages 3-5 of the published final draft of *Guidance for Identifying Pesticide Chemicals and other Substances that have a Common Mechanism of Toxicity,* dated January 29, 1999.

<u>QUESTION 2:</u> “ ...... Determinations of whether a common mechanism of toxicity is operating will be based on all available information evaluated by a weight of evidence approach as demonstrated by the case study. How might this process be improved to make it more scientifically sound?”

The SAP noted: The Panel strongly endorses the weight of evidence approach for determining presence or absence of common mechanism of action. The Panel encourages the Agency to incorporate peer reviewed scientific publications into the weight of evidence. Additional scientific evidence can be incorporated provided it is reliable and reproducible. Anecdotal information should not be used.

The Agency responded by including the following lines: “.... Thus, identification of toxic mechanisms will involve an initial search of Agency databases and the literature (step 3a, Figure 1) for assessments or studies that describe mechanisms of toxicity for any of the pesticides grouped in step 2. The types of literature sources that will be searched and used include standard reference and text books, **peer-reviewed journals,** government reports, and study reports submitted to the Agency.....” in pages 12 and 13 of the published final draft of *Guidance for Identifying Pesticide Chemicals and other Substances that have a Common Mechanism of Toxicity,* dated January 29, 1999.

<u>QUESTION 3:</u> Two basic strategies will be employed to determine whether a common mechanism of toxicity is operating.

> **a. Structure based: Begin with a group of pesticide chemicals that have one or more structural similarities. Proceed with an investigation of metabolism of the compounds. Determine whether there is a common biological effect. Ascertain the mechanism by which the effect is produced if information is available to do so. Judge whether the mechanism is common to chemicals in the group. Combine risks for those with a common mechanism.**
>
> **b. Mechanism based: Determine the mechanism of individual pesticide chemicals that are or are not structurally related. Combine risks for those that have a common**

mechanism.

Comment on the merits of these two strategies to identify pesticide chemicals for inclusion in combined risk assessment.

The SAP noted that: ".... Chemicals with similar structures may not have the same mechanism of action. However, grouping chemicals with structural similarities can serve as a starting point or a convenient way to triage chemicals for examining the basis for common mechanisms. ....."

The Agency responded by specifying that only .." the initial, preliminary grouping of substances..." may be done using structural similarities and that "....that substances (including any metabolic precursors) identified under this step will not be included in a cumulative risk assessment if it is determined that they do not cause a common toxic effect by a common mechanism..." in pages 7-9 of the published final draft of *Guidance for Identifying Pesticide Chemicals and other Substances that have a Common Mechanism of Toxicity,* dated January 29, 1999.

<u>QUESTION 4:</u> The case study for chloroacetanilides groups pesticide chemicals according to three grouping scenarios based upon varying degrees and quality of evidence ...... Are these groupings consistent with the Agency's proposed methodologies and definitions?

The SAP noted that: The case study provided by the Agency on an approach for determining common mechanism of action was excellent, well-presented, and very appropriate. The Panel suggests that the Agency develop an equally illustrative example for determining a common mechanism of action for a group of chemicals with a non-cancer endpoint.

The Agency is at present studying the possibility of developing case studies using non-cancer endpoints with the pyrethroids and with compounds related to triclopyr

<u>QUESTION 5:</u> The Agency recognizes that scientific judgment is critical to the determination of whether a mechanism of toxicity has been identified and whether a mechanism is common across chemicals. To ensure that Agency decisions are based on good scientific principles, at least initially, individual assessments on chemical classes will be subjected to external peer review. Comment on the adequacy of this plan in ensuring high scientific standards.

The SAP noted: " .....The Panel strongly endorses the use of peer review to support or reject the Agency's position regarding a common mechanism of action for selected groupings of chemicals ....."

The Agency includes the statement: "Peer review of EPA's decisions concerning: utilization of established toxic mechanisms; identification of toxic mechanisms for specific substances; and grouping (or non-grouping) of substances for purposes of cumulative risk assessment will be solicited in situations in which the Agency believes additional evaluation is needed to ensure that Agency decisions are consistent, well-reasoned and reflect current scientific thinking. " on page 14 of the published final draft of *Guidance for Identifying Pesticide Chemicals and other Substances that have a Common Mechanism of Toxicity,* dated January 29, 1999.

7. Indicate the date the Management approved the Evaluation Comments.
The evaluation comments were approved by Management on January 29, 1999.

8. Have expenses related to the peer review been incurred? If so, please indicate costs and the sources of funding..
The cost of convening this SAP was approximately $5000. This included the room rental cost and travel expenses of the chairman and *ad hoc* members. This funding comes from the Health Effects Division's budget, a portion of which is set aside specifically for SAP reviews. Total FTE costs for this peer review were approximately 0.1 FTE.